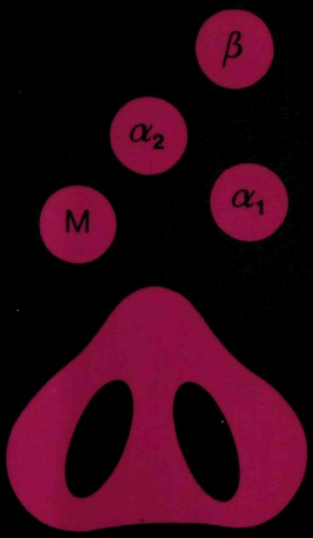
Neuroreceptors in nasal allergy

Y.J.B. van Megen



NEURORECEPTORS IN NASAL ALLERGY

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een wetenschappelijke proeve op het gebied van de
Geneeskunde en de Tandheelkunde

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Ut quimus, aiunt, quando ut volumus non licet.

Terentius, Andria, 4,5, 10(805).

CONTENTS

Nasal hyperreactivity can best be defined as an increased response of a nasal effector system to a stimulus. The clinical symptoms (increased response) are sneezing, hypersecretion and nasal obstruction. Allergens are the stimuli for specific nasal hyperreactivity, described as atopic or allergic rhinitis. Allergic rhinitis and allergic asthma occur often simultaneously and have a similar pathophysiology, indicating that both manifestations are the same hyperreactivity in the upper and lower airways respectively. Hyperreactivity of the lower airways has been explained by various mechanisms; the theory of Szentivanyi (1968), in which dysfunction of the β-adrenoceptors has been postulated, has received particular attention. Other studies (Barnes et al., 1980; Raaijmakers et al., 1984) support the imbalance of the autonomic nerve regulation in bronchial hyperreactivity by differences in neuroreceptor densities. Analogous to the bronchial hyperreactivity, nasal hyperreactivity may be explained by an imbalance of the autonomic nerve regulation. Until now, histochemical and pharmacological experiments have only given a general impression of the innervation of the nasal mucosa, but give no any information on the characterization or localization of the various neuroreceptors. In contrast with the lower airways, only a few quantitative data (densities) of neuroreceptors in homogenates of the nasal mucosa are available (Ishibe et al., 1983).

In this study radioligand receptor binding and in vitro autoradiographic studies were performed to investigate biochemical characteristics, densities, sensitivities, subclasses, receptor-effector coupling or localization of the neuroreceptors in the nasal mucosa. The rat was used as an experimental model, so that the restricted amount of human nasal mucosa was not a limiting factor. Both techniques have been applied to nasal mucosa of non-allergic and allergic patients, in order to investigate the supposed changes in characteristics of neuroreceptors in nasal hyperreactivity. The non-allergic group has been further subdivided into control, vasomotor rhinitis and chronic sinusitis patients. A histological study of the nasal mucosa of both groups was involved for the interpretation of the receptor binding studies.

AA = arachidonic acid
AB/PAS = Alcian Blue/Periodic Acid Schiff
ADP = adenosine 5'-diphosphate
ATP = adenosine 5'-triphosphate
Bmax = receptor density
BSA = bovine serum albumin
C = catalytic unit
cAMP = adenosine 3'5'-cyclic monophosphate
cGMP = guanosine 3'5'-cyclic monophosphate
CO = cycloxygenase pathway
CYP = cyanopindolol
D = drug
DG = diacylglycerol
EDTA = ethylenedinitrilo tetra-acetic acid disodium salt
ER = endoplasmic reticulum
$G\alpha\beta\tau$ = G-protein with subunits
GC = guanylate cyclase
Gpp(NH)p = 5'-guanylylimidodiphosphate
GTP = guanosine 5'-triphosphate
H = agonist
HAA = hydroperoxide of arachidonic acid
HEPES = 4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acid
HEPES-PI = HEPES buffer with protease inhibitors
HHSiD = hexahydrosiladifenidol
IgE = immunoglobulin E
IP_3 = inositoltrisphosphate
IP_4 = inositoltetrakisphosphate
Kd = equilibrium dissociation constant
Ki = inhibition constant
LO = lipoxygenase pathway
MC = mast cells
PG = prostaglandin

PI = posphatidylinositol

PIP_2 = phosphatidylinositol 4'5'-biphosphate

PK = protein kinase

PKC = protein kinase C

PL = phospholipid

PLC = phospholipase C

PLA_2 = phospholipase A_2

PMSF = phenylmethylsulfonylfluoride

QNB = quinuclidinylbenzilate

R = receptor

RAST = radioallergosorbent test

SEM = standard error of the mean

SP = substance P

$t_{½}$ = half life time

TAME = Nα-p-tosyl-L-arginine methyl ester

Tris = Tris(hydroxymethyl)aminomethane

Tx = thromboxane

LT = leukotriene

VIP = vasoacive intestinal polypeptide

INTRODUCTION

(partially published in O.R.L. 50: 32, 1988)

1.1. Anatomy and physiology of the nose.

The nose has a function in the filtration, humidification and thermoregulation of the inspired air. The nasal fluid, mainly a secretory product of the nasal mucosa, is involved in the humidification of the inspired air, the removal of the inhaled pollutants and the protection of the underlying respiratory epithelium. The vascularization of the nasal mucosa has mainly a function in the warming of inhaled air and regulating the patency of the nasal passages (Mygind, 1978, Drettner, 1979, Proctor, 1982, Geurkink, 1983).

The nasal cavity is divided into two identical cavities by the nasal septum. In each of these cavities three regions with different functions can be distinguished; the vestibular, the respiratory and the olfactory. The vestibular region is restricted to a narrow zone inside the nostrils. The olfactory region comprises the lateral and dorso-medial part of the nasal cavity. The remaining part of the nasal cavity is occupied by the respiratory region, this being the aim of the study.

In the respiratory region some components can be distinguished in the nasal mucosa; the epithelium and the lamina propria with the glands, blood vessels, inflammatory cells and nerves (Mygind, 1978; Proctor, 1982; Klaassen et al., 1988). The anatomy of these structures is species dependent; the anatomy and physiology of the rat nasal mucosa will be described and the differences between the rat and human nasal mucosa.

1.1.1. The epithelium.

In the respiratory region, the epithelium is resting on a basement membrane. This membrane consists of a thin continuous double membrane on a connective tissue membrane (thick layer of collagen with reticulin

fibrils). The typical ciliated, columnar pseudostratified respiratory epithelium consists of 4 cell types (Rhodin, 1974; Mygind et al., 1982a)(fig. 1.1):

I The basal cells.

These cells are very small and do not reach the luminal surface. The other cell types originate from these undifferentiated cells.

II Goblet cells.

Part of the goblet cells may originate from the ciliated cells and not from the basal cells (Bauer and Temesrekasi, 1967). Goblet cells are unicellular glands, which can aggregate to form intra-epithelial glands. The goblet cell is characterized by a well developed Golgi complex and a somewhat irregular apical part without microvilli. In the secretory active cells, the nucleus is pushed toward the basal part of the cell by the numereous apical localized secretory granules. The cell protrudes slightly into the lumen. After the extrusion of the secretory granules, the apical cell membrane recloses (Mogensen and Tos, 1976).

III Non-ciliated columnar cells.

These cells are covered with microvilli. Microvilli cannot move actively. They increase the surface area of the epithelial cells, thereby promoting the transport of substances and water between cells and the nasal fluid.

IV Ciliated columnar cells.

These cells are covered with both cilia and microvilli. Cilia are long thin mobile projections from the luminal surface of the cell. The characteristic cross section of a cilium is a ring of nine doublet microtubules surrounding two single central microtubules. Dynein arms are present on one side of each of the nine doublets. Dynein, with ATP-ase activity, is thought to be responsible for the active ciliary movement. The cilia together with the mucous layer have a cleansing function by moving debris and bacteria to the nasopharynx.

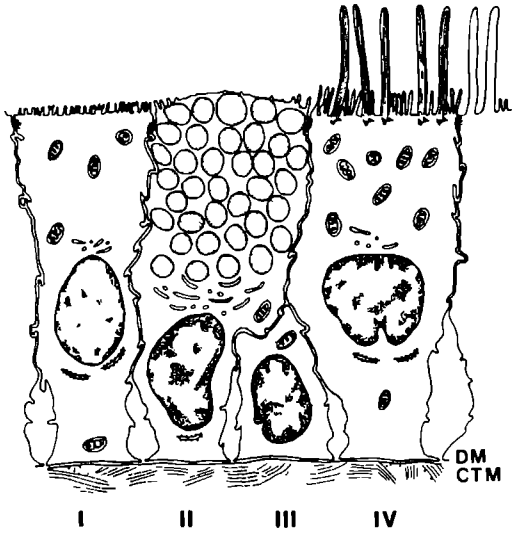


Fig. 1.1:
Transmission electronic microscopic diagram of the four cell types in nasal respiratory epithelium.
I Non-ciliated columnar cells
II Goblet cells
III Basal cells
IV Ciliated cells
DM= Double membrane
CTM=Connective tissue membrane
(Mygind, 1978; with permission)

1.1.2. Lamina propria.

The lamina propria or submucosa consists of connective tissue (fibroblasts, fibrocytes, histiocytes, fibrils), glands, blood vessels, inflammatory cells and nerves.

1.1.2.1. The glands.

The glands of the respiratory area may be divided into goblet cells (secretory cells of the epithelial lining) and submucosal glands. The nasal fluid is mainly a secretory product of the nasal glands but it is also a mixture of transudate, tears, condensed water of the expired air, loose cells and micro-organisms (Mygind, 1978). Biochemical analysis revealed the presence of many chemical substances such as serum proteins, immunoglobulins, enzymes and glycoproteins (Widdicombe and Wells, 1982). Glycoproteins in particular are responsible for the viscosity and gelforming properties of the mucus (Clamp et al., 1978). The acini of the glands produce the glycoproteins and it has been suggested that the glandular striated excretory ducts control the ion and water content of the secretion (Phipps, 1981).

The submucosal glandular structures of rodents are concentrated in two large masses; one in the lateral wall and one in the septum (Bang and Bang, 1959; Bojsen-Møller, 1964; Vidic and Greditzer, 1971, Klaassen et al., 1981). Two glandular parts can be distinguished with regard to their duct system (fig. 1.2.), although there is no clear anatomical boundary (Klaassen et al., 1981). The dorsal part, described as the maxillary glands (Bang and Bang, 1959; Vidic and Greditzer, 1971) has no typical intercalated or striated excretory ducts and drains by some simple excretory ducts into the lumen of the maxillary sinus. The ventral part, described as the lateral nasal gland by Vidic and Greditzer (1971), consists of very branched tubulo-acinous glandular tissue and many intercalated ducts. From this ventral part, one large excretory duct courses towards the vestibulum in addition to a number of smaller excretory ducts (Klaassen et al., 1981). Bojsen-Møller (1964) considered this ventral part as 2 anatomically separated glands, the anterior and the Steno lateral glands.

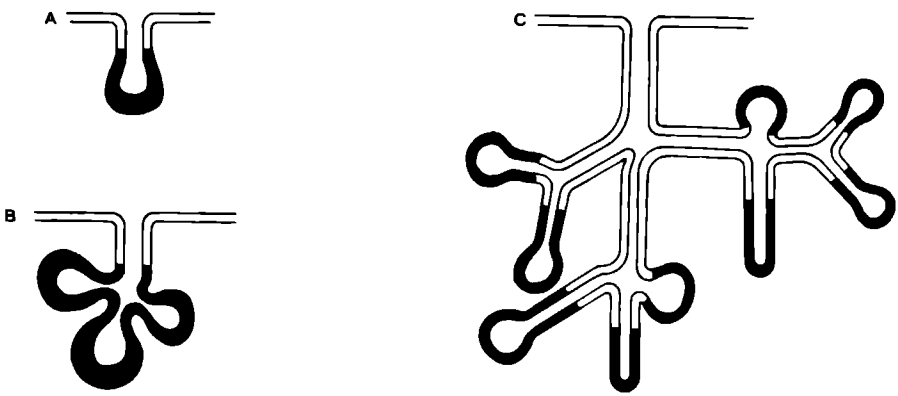


Fig. 1.2: Simple and compound multicellular glands in human (A/B) and rat (B/C) nasal mucosa. The glandular cells are black and the non-glandular cells are clear. A=simple acinar (alveolar) B=simple branched acinar (alveolar) C=compound tubulo-acinar (Klaassen et al., 1981; with permission).

Using histochemical techniques for glycoproteins three different areas (L_1 and L_3 in the ventral part and L_2 in the dorsal part) may be distinguished; the dorsal part containing neutral and sulphated glycoproteins and the ventral part neutral and sialyted (Klaassen et al., 1981). These areas may also be distinguished by different granules in their acinous cells (Klaassen et al., 1982). There are also differences in the secretory behaviour of the histochemically different dorsal and ventral rat nasal glands. The ventral part has a higher sensitivity in glycoprotein secretion on stimulation with the cholinergic agonist metacholine, possibly due to differences in neural regulation (Klaassen and Kuijpers, 1986a) (Klaassen et al., 1987).
The glandular tissue of the rat nasal septum contains one large gland with a moderate developed duct system (5 large excretory ducts) between the acini and may be considered as a branched tubulo-acinous gland. Two different areas may be distinguished on the basis of various glycoproteins and secretory granules in their acinar cells (Klaassen et al, 1981; Klaassen et al., 1982).

Large glandular systems, as present in the lateral wall of the rat nasal mucosa, are only found in human embryos and then regress completely during subsequent development (Bast, 1924). In man the submucosal glands may be divided into two groups; the anterior and the tubulo-alveolar glands. The anterior glands open into the crypts in the region of the internal ostium. The tubulo-alveolar glands are scattered throughout the respiratory area and drain by small excretory ducts which frequently end with a funnel-shaped opening (Bojsen-Møller, 1965; Tos, 1976). Ultrastructural studies reveal that striated excretory ducts are absent in these glands (Jahnke, 1972). Using various histochemical techniques; neutral, sulphated and sialyted glycoproteins have been detected in acinar cells of the acini (Thaete et al., 1981).

1.1.2.2. The blood vessels.

The vascularization of the nasal cavity has a crucial function in the warming of the inspired air and in the regulation of the patency of the nasal passages by means of venous erectile tissue.

In the rat the internal carotid artery gives off the pterygopalatine artery, which has two branches: a pterygoid and a palatine branch. The artery of the pterygoid canal (vidian artery) is a continuation of the pterygoid branch of the pterygopalatine artery. This artery runs forwards on the lateral surface of the internal pterygoid and enters the posterior nares to supply the nasopharynx and the inferior part of the nasal cavity. The palatine portion gives off two branches; the ophthalmic and the sphenopalatine. The ophthalmic artery gives rise to the ethmoidal artery, which runs through the dorso-anterior part of the nose through the ethmoidal foramen. The sphenopalatine artery, which supplies the main part of the mucosa of the septum and the lateral wall, enters the nasal cavity through the sphenopalatine foramen. One of its branches has been shown to anastomose with the descending palatine artery by way of the incisive canal (Greene, 1959; Grote, 1974).

In man the blood supply to the nasal mucosa comes from both the internal and external carotid artery (Dankmeyer, 1964; Osborn, 1978):

a. from the external carotid artery via the sphenopalatine artery to the turbinates, lateral walls and inferior parts of the septum.
b. from the internal carotid artery via the anterior and posterior ethmoidal arteries to the superior parts of the the nose.

In both man and rat nasal mucosa the capillaries are situated at three levels (Mygind, 1978; Cauna, 1982): subepithelial, periglandular, perichondral and periosteal. Nasal fenestrated capillaries are characterized by the absence of an internal elastic membrane (Cauna and Cauna, 1975) and by the porosity of the endothelial basement membrane (Watanebe and Watanebe, 1980).

Between the capillaries and the venules are interposed the cavernous sinusoids, localized in the basal part of the lamina propria. The sinusoids are regarded as specialized capillaries and are normally in a contracted condition. Filling of the sinusoids with blood influences nasal patency, resulting in a congestion of the mucosa. There are also direct connections between arterioles and venules; the arteriovenous anastomoses (Cauna, 1982).

The blood vessels can be classified according to their principal function into capacitance, resistance and exchange vessels (Anggård,

1977; Mygind, 1978; Cauna, 1982) (fig. 1.3.). The tone of the capacitance vessels (mainly venous vessels and sinusoids) regulates the blood volume, whereas the tone of the resistance vessels (mainly small arteries, arterioles and arteriovenous anastomoses) regulates the blood flow. Transport through the wall takes place in the exchange vessels (mainly capillaries).

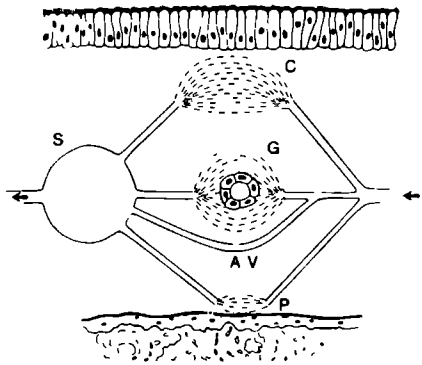


Fig. 1.3.:
The vascular arrangement in the nasal mucosa. A-V=arteriovenous shunt vessel, C=subepithelial capillaries, G=Periglandular capillaries, P=perichondreal capillaries, S=Venous sinusoids (Anggård, 1977; with permission).

1.1.2.3. Infiltrating cells.

In the lamina propria many infiltrating cells are present; leucocytes, lymphocytes, plasma cells, macrophages and basophilic cells. The basophilic cells may be subdivided into blood basophilic leucocytes and tissue mast cells. The basophilic leucocytes have distinct granulocyte characteristics, including polylobed nuclei with condensed chromatin, cell size ranges between 7 and 10 μm with irregular blunt or thick surface processes, cytoplasmic glycogen and granules generally larger and fewer in number than those in mast cells (Galli et al., 1984).

Two distinct types of mast cells have been identified by their morphologic and functional properties; the connective tissue mast cells and the mucosal mast cells (Bienenstock, 1988). The common characteristics include cell size (ranges between 8 and 20 μm), surface IgE receptors, numereous cytoplasmic granules (about 200), lipid bodies, cytoplasmic filaments and an excentric nucleus (Galli et al., 1984). Activation of mast cells leads to release of the mast cell mediators from

the granules. Degranulation is an active secretory process requiring energy and Ca^{2+}. The mediators may be divided into (Robinson and Holgate, 1985; Wasserman, 1987):

- vasoactive and smooth muscle mediators.
  (histamine, platelet activating factor and arachidonic acid metabolites; PGD_2, TxA_2, LTC_4, LTD_4 and LTE_4).
- chemotactic mediators.
  (eosinophil chemotactic factor, neutrophil chemotactic factor).
- structural proteoglycans.
  (heparin, chondroitin sulphate).
- granule associated enzymes.
  (chymase, tryptase, kallikrein, β-exoglycosidase).

The mediators of the arachidonic acid metabolites consist of the cyclo-oxygenase products, prostaglandins (PG) and thromboxane (TxA), and of the lipoxygenase products, leukotrienes (LT) (Mygind, 1986).
The mast cell release is inhibited by β-adrenergic agonists, histamine and PGE_2 and facilitated by α-adrenergic and cholinergic agonists and by $PGF_{2\alpha}$ (Mygind, 1986; Warner et al., 1988).

1.1.2.4. Nerves.

The nasal mucosa is supplied from the sensory and the autonomic nervous system (parasympathetic and sympathetic)(Christensen, 1934; Grote, 1974; Grote et al., 1975; Ishii and Toriyama, 1972). The sensory fibres are thought to come from the trigeminal nerve. The sympathetic fibres are derived from the superior cervical ganglion. The postganglionic sympathetic fibres run in the plexus of the carotid artery, branch off in the bony carotid canal and form the deep petrosal nerve. Subsequently, the deep petrosal nerve joins the greater petrosal nerve to form the vidian nerve, which reaches the nose via the pterygopalatine fossa. The parasympathetic fibres arise in the superior salivary nucleus in the brainstem and course to the vidian nerve as the greater petrosal nerve. The parasympathetic fibres synapse in the pterygopalatine ganglion. Recently, it has been suggested that the parasympathetic pathway has its postganglionic neurons not only in the pterygopalatine ganglion but also in so-called microganglia, located within the mucosa of the inferior

turbinates (Galan Cortes et al., 1986).

1.2. Sensory and autonomic innervation of the nasal mucosa.

The nasal mucosa is innervated by autonomic (sympathetic and parasympathetic) nerve fibres and also by sensory nerve fibres. The classical neurotransmitters noradrenaline and acetylcholine occur in the sympathetic (adrenergic) and parasympathetic (cholinergic) fibres. Apart from these classical neurotransmitters, peptidergic transmitters, e.g. vasoactive intestinal polypeptide (VIP) and Substance-P (SP), have been identified and localized. Other neuropeptides (avian pancreatic polypeptide, neuropeptide-Y, peptide histidine isoleucine) have also been described, but the physiological significance of these peptides is unknown (Uddman et al., 1978; Uddman and Sundler, 1979; Uddman et al., 1980).
The innervation pattern of the nasal mucosa has been investigated by histochemical, electrophysiological and denervation experiments (Anggård et al., 1972; Ishii and Toriyama, 1972; Anggård et al., 1974; Grote, 1974; Grote et al., 1975; Cauna, 1982; Vecerina et al., 1983). Pharmacological studies have contributed to a deeper insight into the character of the autonomic innervation and its functional significance (Proctor and Adams, 1976; Eccles, 1982; Mygind, 1982a, Malm, 1983).

1.2.1. Glands.

Histochemistry.
Periacinar acetylcholinesterase containing fibres have been identified in the nasal glands. No cholinergic fibres were found in relation to the excretory ducts. No adrenergic innervation of the glands could be demonstrated. Interconnections between the cholinergic periacinar plexus and the vascular plexus have been observed.
VIP and SP immunoreactive neurons have been demonstrated in nasal glands. Anggård (1983) demonstrated that the VIP-reactive neurons represent postganglionic parasympathetic ones and he suggested an interaction between the sympathetic neurotransmitter and this peptide.

Pharmacology.
Electrical stimulation of the parasympathetic nerve supply induces nasal secretion, which can be completely inhibited by atropin (Eccles and Wilson, 1973). Parasympathomimetics stimulate the discharge of granules from rat glandular cells (Klaassen and Kuijpers, 1986a). In clinical trials the parasympatholytic drug ipratropium has been used to reduce nasal hypersecretion (Borum et al., 1979). It has been suggested that α-adrenoceptors play a role in nasal secretion (Malm, 1983), however, a reduction of nasal secretion with α-adrenergic agonists has also been reported (Phipps, 1981). This controversial data may be due to the techniques used and the complexity of origin of the nasal fluid.
The neuropeptide VIP has a stimulatory effect on the secretory activity of the nasal glands (Lundberg, 1981). The effect of SP on the nasal glands is unknown but it is observed that SP stimulates the secretion in rat parotid and submandibular glands (Pernow, 1983).

1.2.2. Blood vessels.

Histochemistry.
Noradrenaline- as well as acetylcholinesterase-containing fibres have been found in the walls of the nasal vessels. The sinusoids, especially, have a very dense network of both fibres, regulating the swelling and shrinkage of the nasal mucosa (Grote et al., 1975; Anggård and Densert, 1974).
VIP and SP containing nerve fibres have been identified in the nasal blood vessels (Uddman et al., 1980b; Uddman et al., 1983)

Pharmacology.
Parasympathomimetics provoke a vasodilatation, which may be blocked by atropine. Electrical stimulation of parasympathetic nerves also causes a dilatation, which is partly inhibited by atropine (Malm, 1973; Eccles and Wilson, 1974). The atropine-resistant vasodilatation might be due to bradykinin or VIP (Lundberg, 1981). The sympathetic innervation of the nasal blood vessels is dual, α-adrenergic agonists induce a vasoconstriction (Jackson, 1980), whereas β-adrenergic agonists induce a

vasodilatation (Hiley et al., 1978; Malm, 1977). The significance of α-adrenoceptors for the control of the blood volume as well as blood flow has been verified (Andersson and Bende, 1984; Bende, 1985), on the other hand β-adrenoceptors have been suggested to be of minor functional importance (Hall and Jackson, 1968; Svensson et al., 1980). Clinically treatment of pathological nasal congestion may be obtained with drugs affecting the adrenergic system (Bende, 1983).
Both VIP and SP induce vasodilatation (Lundberg, 1981; Lundblad et al., 1983). Recently, it has been reported that neuropeptide-Y, which induces vasoconstriction, coexists in the sympathetic nerves (Ichimura et al., 1988).

1.2.3. Epithelium.

Histochemistry.
The (sub)epithelial plexus gives a weak acetylcholinesterase and noradrenaline reaction (Klaassen et al., 1988). Electron microscopic observations show the presence of unmyelinated axons between the epithelial cells. These axon endings are situated either superficial to the basement membrane or close to the airway lumen beneath the tight junctions (Gamse et al., 1980).
Sub-P containing nerves have been detected beneath and sometimes within the surface epithelium (Uddman et al., 1983).

Pharmacology.
The functional significance of the (sub)epithelial plexus is not yet elucidated. In comparing data on this plexus in the lower airways, it seems likely that one part of these axons may be considered sensory and the other part may be efferent adrenergic and possibly cholinergic (Jeffrey and Reid, 1973; Gamse et al., 1980). The sensory nerves are involved in the nasal reflexes; respiratory, vasomotor and cardiac reflexes (McCaffrey, 1983).

1.3. Neuroreceptors.

Intercellular communications in multicellular organisms is achieved by interactions of specific substances, e.g. hormones and neurotransmitters, released from one cell interacting with a specific receptor protein on a target cell. This interaction results in one or more changes in the activity of an intracellular or plasma membrane protein in the target cell, which modifies physiological responses.

It has been suggested that neuroreceptors consist of at least two components, the binding site and a site which translates the transmitter recognition into a biochemical effect (Birdsall, 1984; Levitzki, 1986; Nathanson, 1987). The neuroreceptors have been studied in physiological, pharmacological, radioligand receptor binding studies and autoradiographic experiments (Laduron, 1984).

Different molecular models have been proposed for the interaction of the neurotransmitter or drug with the receptor; e.g. the occupation model, the rate theory, the two state model and the mobile receptor model (Hollenberg, 1978; Ariens et al., 1979). In this study equations based on the occupancy theory were used. This theory assumes a bimolecular reaction of one drug with one receptor, at equilibrium with a dissociation constant (Kd) given by

$$Kd = \frac{[D][R]}{[RD]} \quad \text{based on} \quad R + D \rightleftarrows RD \longrightarrow E$$

in which [D] = free concentration of drug
[R] = free concentration of receptor
[RD] = concentration RD-complex
E = effect

Furthermore, the theory is based on a direct proportionality between the concentration of the receptor-drug-complex and the pharmacological effect.

In pharmacology, the nervous system may be subdivided on the basis of different neurotransmitters, such as the cholinergic, adrenergic, histaminergic and dopaminergic system. Every system has characteristic neuroreceptors with a certain homogeneity; it has been suggested that these receptors may be derived from a common parent system (Topiol, 1987;

Venter et al., 1988) or common parent genes (Hall, 1987). In this chapter the different types of neuroreceptors and their biochemical coupling to the effector systems will be described.

1.3.1. Biochemical coupling to effector systems

In the biochemical coupling of receptors to the effector systems coupling proteins, G-proteins are often involved. The effector systems may comprise the adenylate cyclase, PI metabolism, guanylate cyclase and ion channels. One type of neuroreceptor can regulate more effector systems. The situation is even more complex, since many actions of the effector systems can interfere with other second messenger systems (Burgoyne et al., 1987). An overview of the distinct G-proteins, effector systems and their interactions is discussed below and illustrated in fig 1.4.

1.3.1.1. G-proteins.

G-proteins, or N-proteins, are intermediates in transmembrane signalling pathways. The G-proteins are heterotrimers with α-, β- and τ-subunits. The α-subunit appears to be the primary effector of the system, it contains the GTP binding site and it exhibits GTP-ase activity. The $\beta.\tau$-subunits play a role in the interaction with the receptor and G-protein stabilization, and are shared by various G-proteins. One receptor can interact with more than one G-protein (Kenakin, 1988) and different G-proteins are involved in different effector systems (Litosch, 1987; Levitzki, 1987; Graziano and Gilman, 1987; Dolphin, 1987; Dunlap et al., 1987):

- G_s and G_i proteins are associated with adenylate cyclase activation and inhibition respectively. The regulation of the catalytic unit by the two G-proteins is not symmetric; G_s is tightly bound to the unit, whereas G_i has not been shown to form a complex, and G_i is usually present in much higher amounts than G_s.
- Gp proteins are associated with activation of phospholipase C, resulting in an increased PI-turnover.
- Go proteins are associated with receptor mediated regulation of ion

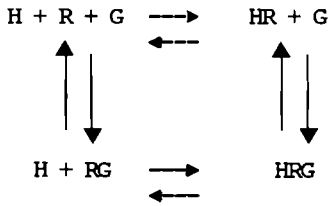
channels.

- G-proteins associated with activation of phospholipase A_2.

1.3.1.2. Adenylate cyclase.

Activation of neurotransmitter receptor sites enhance or reduce adenylate cyclase activity, catalysing the production of cAMP from ATP. In both cases the receptor signal is transmitted to the catalytic unit of adenylate cyclase through G-proteins (see 1.3.1.1.). The second messenger cAMP activates a kinase that catalyses the phosphorylation of cellular proteins and is converted to 5'-AMP by phosphodiesterase (Stiles et al., 1984; Enna and Karbon, 1987).

The initial event of receptor stimulation is presumably the binding of the agonist (H) to the receptor (R) to form the low affinity HR-complex (fig. 1.5). Binding of this complex to G-proteins results in a ternary complex (HRG), representing the high affinity state of the receptor. However, it is also possible that the receptor is firstly coupled to the G-protein, followed by binding of the agonist (Stiles et al., 1984; Levitzki, 1987; Neubig et al., 1988).



The ternary complex induces a conformational change in the α-subunit, increasing GDP exchange for GTP. Two consequences ensue after the Mg^{2+}-dependent GTP binding to the G-protein. First, the R-G complex is destabilised, resulting in a conversion of the high affinity state to the low affinity state, and second the G-protein is dissociated into its subunits. The subunit α-GTP complexes probably with the effector system. The activated α-subunit has GTP-ase activity and becomes deactivated when GTP is hydrolysed. The α-GDP unit reassociates with the $\beta.\gamma$-unit (Stiles et al., 1984; Dolphin, 1987; Vauquelin et al., 1988).

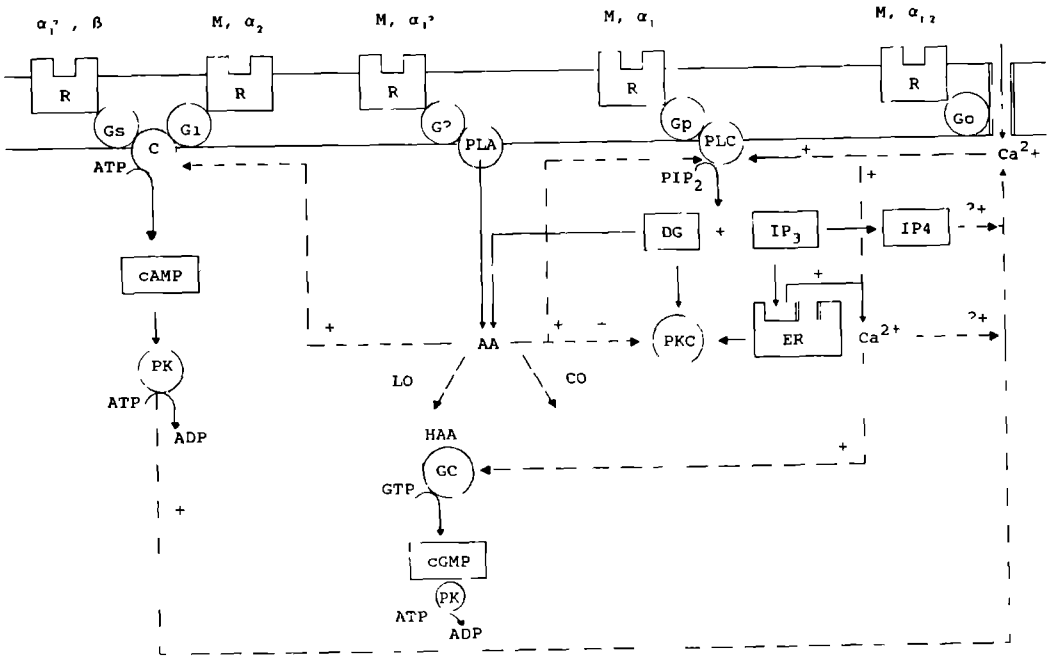


Fig. 1.4.:
Biochemical coupling of various neuroreceptors to effector systems.
α=α-adrenoceptors, AA=arachidonic acid, ADP=adenosine 5'-diphosphate, ATP=adenosine 5'-triphosphate, ß=ß-adrenoceptor, C=Catalytic unit, cAMP=adenosine 3'5'-cyclic monophosphate, cGMP= guanosine 3'5'-cyclic monphosphate, CO=cyclooxygenase pathway, DG=diacylglycerol, ER=enoplasmic reticulum, G=G-protein, GC=guanylate cyclase, GTP=guanosine 5'-triphosphate, HAA=hydroperoxide of AA, IP_3=inositoltrisphosphate, IP_4= inositoltetrakisphosphate, LO=lipoxygenase pathway, M=muscarinic receptor, PIP_2=phosphatidylinositol 4'5'-biphosphate, PK=protein kinase, PL=phospholipid, PLC=phospholipase C, PLA_2=phospholipase A_2, R=receptor, +=stimulation, -=inhibition, ▭ =second messenger.

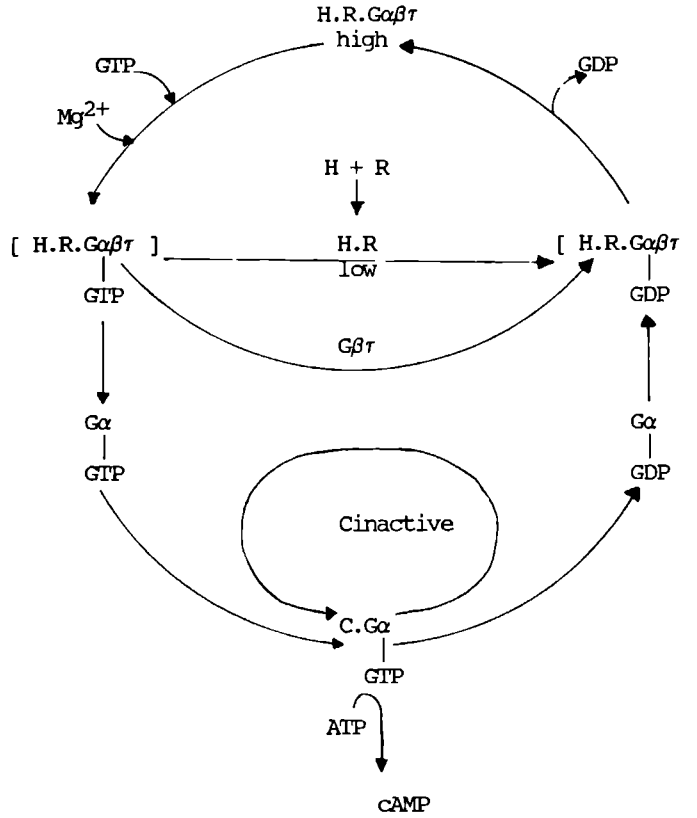


Fig. 1.5: Adenylatecyclase regulation of the neuroreceptor by agonist, G-protein and GTP. ATP=adenosine 5'-triphosphate, C=catalytic unit, cAMP=adenosine 3'5'-cyclic monophosphate, Gαβτ=G-protein with subunits, GDP=guanosine 5'-diphosphate, GTP=guanosine 5'-triphosphate, H=agonist, R=receptor.

1.3.1.3. Phosphatidylinositol (PI) turnover.

Activation of neurotransmitter receptor sites may enhance PI turnover. The receptor signal is transmitted to the enzyme phospholipase C through the Gp-protein (Cockcroft, 1987). Phospholipase C catalyses the conversion of PIP_2 (phosphatidylinositol 4'5'-biphosphate) to IP_3 (inositoltrisphosphate) and DG (diacylglycerol). The formed second messenger IP_3 liberates Ca^{2+} ions from intracellular stores (endoplasmic

reticulum, calciosomes (Volpe et al., 1988)). There is a specific receptor for IP_3 on the endoplasmic reticulum (ER), which mediates the opening of Ca^{2+} channels in the ER membrane. The action of IP_3 is terminated by dephosphorylation of the free inositol or by a kinase mediated phosphorylation forming IP_4. IP_4 may have a second messenger function in regulating the influx of Ca^{2+} ions (Minneman, 1988b; Timmermans, 1988). The Ca^{2+} activated phosphorylase C may represent a positive feedback mechanism for Ca^{2+} (Eberhard and Holz, 1988). The second messenger DG stimulates protein kinase C and produces arachidonic acid metabolites, which may interact with other effector systems (see 1.3.1.4.) (Burgoyne et al., 1987). Protein kinase C, activated by DG but also by Ca^{2+}, modifies cellular activity by catalysing the phosphorylation of various substrates (Taylor and Merrit, 1986; Kaczmarek, 1987; Fisher and Agranoff, 1987).

1.3.1.4. Guanylate cyclase.

Activation of neurotransmitter receptor sites may enhance phospholipase A_2 activity. The receptor signal may be transmitted through G-proteins. Phospholipase A_2, a calcium activated enzyme, catalyses the release of arachidonic acid from membrane phospholipids. Arachidonic acid activates phospholipase C, protein kinase C (see 1.3.1.3.) and has been shown to stimulate adenylate cyclase (see 1.3.1.2.) and guanylate cyclase. Guanylate cyclase can also be activated by Ca^{2+} ions (Burgoyne et al., 1987; Enna and Karbon, 1987) and catalyses the production of cGMP from GTP. The second messenger cGMP activates a kinase that catalyses the phosphorylation of cell protein.

1.3.1.5. Ion channels.

Activation of receptors can lead to either de- or hyperpolarization, due to the opening or closing of ion channels. Regulation of the ion channels occur in different ways. The receptor may be the ion channel or distinct receptor and channel proteins interact with each other through G-proteins. The binding of the agonist at the receptor may also be followed by an alteration in the activity of an enzyme; the altered level of product may introduce changes in the open or closed state of a number

of channels throughout the cells, most probably by phosphorylation of the channels. Available evidence indicated a direct coupling between receptor, G-protein and K^+ channel or Ca^{2+} channels (Gomperts, 1983; Dunlap et al., 1987), but also an indirect activation of the ion channels by second messengers like protein kinase C (Christie and North, 1988).

1.3.2. Muscarinic acetylcholine receptors.

The neurotransmitter acetylcholine has been shown to act at two pharmacologically distinct receptors, muscarinic and nicotinic receptors. Muscarinic acetylcholine receptors are pharmacologically defined by their selectivity towards the agonist muscarine and the antagonist atropine. These receptors mediate various types of responses in cardiac muscle, in numerous smooth muscles, in exocrine glands and throughout the peripheral and central nervous system (McKinney and Richelson, 1984; Nathanson, 1987).

Various subtypes of the muscarinic receptors have been identified over the past years on the basis of receptor binding and pharmacological experiments. Initially muscarinic receptors were subclassified on the basis of the selectivity profile of pirenzepine; receptors with high affinity for this ligand were identified as M_1 subtypes, whereas the M_2 subtype was characterized by a low affinity (Hammer et al., 1980; Hammer et al., 1982; Birdsall, 1984). The M_1 subtype is mainly found in the central nervous system and sympathetic ganglia; the M_2 subtype is found in the peripheral effector organs of the parasympathetic nervous system and also in parts of the central nervous system. M_1 as well as M_2 receptors may be presynaptic muscarinic receptors involved in a negative feedback mechanism of the release of acetylcholine, norepinephrine or ATP (Langer, 1981; Kilbinger, 1984; Wessler and Diener, 1987).

After the development of several selective antagonists, it became clear that M_1 (Lambrecht et al., 1988) and M_2 muscarinic receptors (Birdsall, 1984) are not a homogeneous class. Muscarinic M_1 receptors with high affinity for hexahydrodifenidol and hexacyclium are termed ganglionic $M_{1\beta}$ and those with low affinity are termed hippocampal $M_{1\alpha}$ (Lambrecht et

al., 1988). The subdivision of M_2 receptors has been demonstrated in functional and radioligand receptor binding studies by cardioselective antagonists, such as AF-DX-116 (Micheletti et al., 1987), gallamine (Clark and Mitchelson, 1976), methoctramine (Melchiorre et al., 1987) and by smooth muscle or gland selective antagonists like 4-DAMP (Barlow et al., 1976) and hexahydrosiladifenidol (HHSiD) (Mutschler and Lambrecht, 1984). The classification of the M_2 receptors is not unanimous but they can be divided into at least two subclasses; M_2(heart) or M_2(glands, smooth muscle) receptors. The M_2(heart) receptors have been termed as $M_{2\alpha}$ (Mutschler and Lambrecht, 1984) or M_{2A} (Eglen and Whiting, 1986) and M_2(gland, smooth muscle) receptors have been termed as $M_{2\beta}$, M_{2B} or M_3 receptors (De Jonge et al., 1986; Ladinsky et al., 1988). AF-DX-116 not only can differentiate between heart and glands but also displays an intermediate affinity for ileum smooth muscle (Hammer et al., 1986; Micheletti et al., 1987; Doods et al., 1987). Therefore a subdivision of M_2 receptors into M_2(heart), M_3 (exocrine glands) and M_4 (smooth muscle) has been proposed by Batink et al. (1987). Other authors demonstrated in ileum smooth muscle the cardiac and glandular muscarinic receptors, suggesting the heterogeneous presence of M_2(heart) and M_2(gland) subtypes (Ladinsky et al., 1988; Giraldo et al., 1987). Recently, the classification scheme of M_1, M_2 and M_3 receptors, based on differential affinity for selective antagonists has been generally accepted (Eglen et al., 1989).

Molecular cloning studies have identified four distinct muscarinic receptors in the rat (Kubo et al., 1986) as well as in man (Peralta et al., 1988). The pharmacological subdivision of M_1 and M_2 receptors is supported by showing that the receptors located in the cerebral cortex and the heart represent distinct gene products (in rat m1 and m2; in man HM1 and HM2). Furthermore, two additional muscarinic subtypes have been identified. In rat the pharmacology of these cloned subtypes (m3 and m4) is unknown (Kubo et al., 1986); in man the other cloned subtypes (HM3 and HM4) resemble the pharmacological defined M_1 and M_2(gland) subtypes (Peralta et al., 1988). The receptor subtypes have also different amino acid sequences, although the receptors share a plasma membrane arrangement with seven hydrophobic transmembranal segments, a long C-

terminal hydrophilic sequence, a shorter N-terminal hydrophilic sequence and a long loop between membrane segments V and VI.

Activation of muscarinic receptors by agonists leads to various biochemical responses (McKinney and Richelson, 1984; Nathanson, 1987):

- inhibition of the adenylate cyclase.

  M_1 as well as M_2 muscarinic receptor subtypes are coupled to the adenylate cyclase.

  On the basis of the agonist binding; muscarinic receptors have been divided into two (high and low) and sometimes into three agonist affinity binding sites (super high, high and low). The complex binding behaviour may be due to interconversion of different states of the receptor and/or the existence of distinct receptor subtypes (Birdsall, 1984; Nathanson, 1987).

- stimulation of the phosphatidylinositol (PI) turnover.

  An increased PI turnover is better correlated to the M_1 subtype (Kunysz et al., 1988; Ashkenazi et al., 1987) but also occurs in M_2 muscarinic receptors (Noronha-Blob et al., 1988; Kunysz et al., 1989).

- stimulation of c-GMP synthesis.

  The precise mechanism of activation of these receptors to stimulate guanylate cyclase has not been unequivocally established. Ca^{2+} ions but also arachidonic acid metabolites (see 1.3.1.4.) have been suggested as possible mediators.

- regulation of transmembranal ion transport (Ca^{2+}, K^+ and Na^+ ions).

  Muscarinic agonists activate K^+ channels in cardiac tissue, glands and some neurons and close those channels in many neurons. Muscarinic agonists increase Ca^{2+} and Na^+ permeability in mammalian smooth muscle, some glands and neurones, and decrease Ca^{2+} permeability in cardiac smooth muscle and in other neurones (North, 1986; Nathanson, 1987). The muscarinic regulation of the ion channels is not fully understood. Activation of M_2(heart) receptors leads to the opening of K^+ channels through an intervening G-protein (Christie and North, 1988). It is possible that Ca^{2+} channels, coupled to M_2(gland), are opened in a similar way (Marty, 1987). On the other hand the indirect activation of ion channels is caused by second messengers (see 1.3.1.5.).

1.3.3. Beta-adrenoceptors.

The sympathetic neurotransmitters have been shown to act at two pharmacologically distinct receptors, α- and β-adrenoceptors. The β-adrenoceptor is defined as a receptor for which the agonist isoprenaline is more potent than epinephrine, which again is more potent than norepinephrine. The β-adrenoceptor activities are specifically inhibited by the antagonist propranolol (Ahlquist, 1948).

Two pharmacologically distinct subtypes of β-adrenoceptors, termed β_1- and β_2-adrenoceptors, have been distinguished on the basis of their relative affinities for the agonist epinephrine and norepinephrine (Lands et al., 1967). The β_1-adrenoceptor binds both agonists with approximately equal affinity, whereas β_2-adrenoceptors have a 30 fold higher affinity for epinephrine. Subsequent to this initial classification various β-adrenergic agonists and antagonists have been developed to distinguish between these subtypes (Minneman and Molinoff, 1980; Milavec-Krizman et al., 1985). The subtypes may be present in the same tissue (Minneman et al., 1979) but β_1-adrenoceptors predominate in heart (force and rate) and adipose tissue (lipolysis), whereas the β_2-adrenoceptors predominate in lung (relaxation), liver and smooth muscle (relaxation). Presynaptic β_1, as well as β_2-adrenoceptors are only negligibly involved in a positive feedback mechanism (Langer, 1981; Starke, 1987). According to Ariens and Simonis (1983) β_1-adrenoceptors are meant for the neurotransmitter noradrenaline and β_2-adrenoceptors for the hormone adrenaline.

Recently, the primary structures of the human β_1- and β_2-adrenoceptors have been deduced from the cloning of their genes and/or cDNA. Certain amino acid sequences of the two receptor proteins are similar and both receptors share a plasma membrane arrangement with seven hydrophobic transmembranal segments (20-25 amino acids), a long C-terminal hydrophilic sequence, a shorter N-terminal hydrophilic sequence and a long loop between membrane segments V and VI (Hall, 1987). The pharmacological subdivision of the β_1 and β_2-adrenoceptors is supported by demonstrating that the receptors are derived from different genes (Frielle et al., 1988).

Agonist stimulation of β_1- as well as β_2-adrenoceptors cause a stimulation of the adenylate cyclase, but they can be independently regulated (Donnell and Wanstall, 1987). This stimulation is mediated through G_s as well as G_i proteins. The ability of the G_i-proteins to interact with a stimulatory action may be caused by changing the G_i(GDP)-C-complex (C=catalytic unit) for G_s(GDP)-C-complex (Levitzki, 1986; Levitzki, 1987; Vauquelin et al., 1988). However, not all β-adrenoceptors are able to bind with the G_s-protein but all β-adrenoceptors induce conformational changes, i.e. a temporary opening of the disulphide bond in the receptor, in the first phase of agonist stimulation (Vauquelin et al., 1988). On the basis of the agonist binding β-adrenoceptors have been divided into high and low affinity agonist binding sites

1.3.4. Alpha$_1$-adrenoceptors

The initial subclassification of α-adrenoceptors was based on their localization; presynaptic α_2-adrenoceptors and postsynaptic α_1-adrenoceptors (Langer, 1974). This anatomical classification appeared to be incorrect since α_2-adrenoceptors are also located postsynaptically (Berthelsen and Pettinger, 1977). Subsequently the α-adrenoceptors have been divided into their subtypes based on their pharmacological profiles (Hoffman et al., 1979; Timmermans and van Zwieten, 1982; Johansson, 1984; Vizi, 1986).

Alpha$_1$-adrenoceptors are pharmacologically defined by their binding selectivity for the agonists phenylephrine, methoxamine, SKF89748, cirazoline and of the antagonists prazosin, BE2254 and corynanthine (Timmermans and van Zwieten, 1982; Hieble et al., 1986; Vizi, 1986). The α_1-adrenoceptors are predominantly postsynaptic sites (Starke, 1987) in smooth muscle, liver, heart, salivary and sweat glands, adipose tissue, kidney and brain. They mediate mainly stimulatory physiological actions (contraction/relaxation, glucogenolysis, positive inotropy/chronotropy,

secretion, Na^+ absorption)(Timmermans, 1988).
Recently, the presence of α_1-adrenoceptors subtypes has been suggested because of differences in the potencies of drugs, differences in affecting contraction of smooth muscle, differences in second messenger formation in liver and brain and differences in inhibiting radioligand binding. The antagonist WB4101 distinguishes high, α_{1A}, and low affinity binding sites, α_{1B} (Morrow and Creese, 1986; Han et al., 1987). The agent chlorethylclonidine was found to inactivate α_1-adrenergic binding sites and functional responses in some tissue. It has been suggested that the chlorethylclonidine-sensitive and -insensitive sites are equivalent to those with a low and high affinity for WB4101 (Minneman et al., 1988a).

Agonist stimulation of α_1-adrenoceptors leads to:
- an increased PI turn over, probably mediated by the α_{1A}-adrenoceptor subtype (Minneman, 1988b; Timmermans, 1988). Generally, a homogeneity of agonist binding to α_1-adrenoceptors has been observed (Agrawal and Daniel, 1985; Guicheney and Meyer, 1981). Recently, several studies indicated high and low, GTP-sensitive agonist binding sites (Terman et al., 1987; Jagadeesh and Deth, 1987).
- directly opening of Ca^{2+} channels (Chiu et al., 1987) probably mediated by the α_{1B}-adrenoceptor subtype (Minneman, 1988b).
- possible stimulation of adenylate and guanylate cyclase. These alternate biochemical responses have been suggested but may be indirect responses of the PI turnover (Minneman, 1988b).

1.3.5. Alpha$_2$-adrenoceptors.

The α_2-adrenoceptors are pharmacologically defined by their binding selectivity of the agonist oxymetazoline, BHT920 and of the antagonists rauwolscine (stereoisomer of yohimbine), idazoxan and RX781094 (Timmermans and van Zwieten, 1982; Vizi, 1986). Radioligand and functional studies indicated that α_2-adrenoceptors are not a homogeneous group with differences in rodents and non-rodents. A definition of the α_2-adrenergic subtypes is proposed on the base of the affinity for prazosin; α_{2A} and α_{2B} receptors having a low and a high affinity

respectively (Bylund, 1985; Nahorski et al., 1985). Molecular cloning studies have identified 2 distinct genes, which may represent the α_2-adrenoceptor subtypes. The deduced amino acid sequence is most similar to the cloned β-adrenoceptors and also to the muscarinic cholinergic receptors (Kobilka et al., 1987).

Postsynaptic α_2-adrenoceptors have been found in vascular smooth muscle (vasoconstriction), platelets (aggregation), fat cells (inhibition lipolysis) and pancreatic islands (inhibition hyperglycemia). Presynaptic α_2-adrenoceptors have been found in noradrenergic nerve endings (e.g. in the pulmonary artery, heart, vas deferens), cholinergic nerve endings (e.g. bladder, stomach, ileum) and serotonergic nerve endings (hippocampus)(Timmermans and van Zwieten, 1982). Presynaptic α_2-adrenoceptors are involved in a negative feedback of the release of the transmitter norepinephrine. The presynaptic receptors are called autoreceptors, if the noradrenergic neuron A inhibits its own norepinephrine (A) release; they are called heteroreceptors if the noradrenergic neuron B inhibits the norepinephrine release from neuron A (Timmermans and van Zwieten, 1982; Timmermans, 1988).

Agonist stimulation of α_2-adrenoceptors results in (Exton, 1982; Timmermans, 1988):

- inhibition of the adenylate cyclase system for postsynaptic and probably also presynaptic α_2-adrenoceptors. On the basis of the agonist binding, α_2-adrenoceptors have been divided into two (high and low) and sometimes three (super high, high and low) agonist affinity binding sites (Michel et al., 1980; U'Prichard et al., 1983).
- regulation of the transmembranal ion transport. Stimulation of presynaptic α_2-adrenoceptors inhibits the inward current of $Ca2^+$ ions via potential-sensitive permeability channels, whereas stimulation of postsynaptic α_2-adrenoceptors promotes the influx of Ca^{2+} ions. It has been suggested that stimulation of presynaptic α_2-adrenoceptors results in a promotion of K^+ conductances (Timmermans, 1988).

No data are available about differences in biochemical responses of the α_2-adrenoceptors subtypes.

1.3.6. Neuroreceptors in the nasal mucosa.

The presence of cholinergic muscarinic receptors has been demonstrated in homogenates of the nasal mucosa of rat (Klaassen et al., 1986b), guinea pig (Ishibe et al., 1985; Konno et al., 1985) and man (Ishibe et al., 1983; Konno et al., 1987a). Muscarinic receptors have been localized in the nasal glands after in vivo administration of ^{3}H-l-QNB, followed by careful dissection and autoradiography (Klaassen et al., 1984).
The presence of adrenergic receptors has been demonstrated in homogenates of the nasal mucosa of guinea pig (Ishibe et al., 1985; Konno et al., 1986; Konno et al., 1987a) and man (Ishibe et al., 1983, Konno et al., 1987a).

1.4. Nasal hyperreactivity.

1.4.1. Definition, classification and terminology.

Reactivity should ideally be expressed in terms of a stimulus-response curve, which is a sigmoid curve for most systems. Generally, hyperreactivity can be described as a shift in the stimulus-response curve. One of the major problems however in studying nasal hyperreactivity is that there are no adequate methods available for measuring nasal responses. However, symptoms of nasal hyperreactivity can best be defined as an increased response of a nasal effector system to a stimulus (Widdicombe, 1983). The increased responses (clinical nasal symptoms) are sneezing, hypersecretion and nasal obstruction (Mygind, 1978; Mygind, 1986). Stimuli may be specific (allergens) or non-specific (cold air, irritants). Allergens consist of pollen, microfungi, house dust mites and animal products (dander and urine).
The terms atopic and allergic rhinitis have been used for specific nasal hyperreactivity. Atopic diseases include allergic rhinitis and allergic astma. The atopic subjects respond to a daily exposure of minute amounts of allergen. In the immediate, type I anaphylactic reaction (Coombs and Gell, 1975) the allergens react with IgE attached to the surface of the

mast cells, the cells degranulate and liberate chemical mediators, responsible for the symptoms (Mygind, 1986). The late reaction in the airways are consequences of the type I reaction (Mygind, 1986).
Allergic rhinitis can be divided into seasonal and perennial allergic rhinitis (Mygind et al., 1985). Seasonal allergic rhinitis is a generally accepted term for hay fever or pollinosis; the symptoms being seasonal and the allergens pollen (Frankland et al., 1985). Perennial rhinitis is not so well defined; the symptoms are not always perennial, the predominant allergen being the house dust mite (Wihl et al., 1985). The prevalence of allergic rhinitis in the adult general population may be estimated at approximately 10%; the prevalence in males being significantly higher than in females. The symptoms may occur between the ages of 5-45 years, but occur especially in the 10-20 year age group (Weeke et al., 1985). There is often a simultaneous occurrence of allergic rhinitis with asthma; about 18% of allergic rhinitis patients also have asthma and about 50% of asthmathic patients have allergic rhinitis. The short interval between the onset of the two diseases in the same individual strongly indicates that both are manifestations of the same reaction (Weeke et al., 1985).

1.4.2. Pathophysiological mechanisms.

The knowledge of the pathophysiological mechanism of allergic rhinitis is still incomplete, but clinical manifestations have been described. Many immunological manifestations in the immediate and late reaction have been observed:
Immediate reaction
- Elevated IgE in the serum and nasal secretions (Mygind, 1978).
- Increase of the number of inflammatory cells; basophilic leucocytes and mast cells (Okuda et al., 1983; Ohtsuka et al., 1986; Ukai et al., 1986; Gomez et al., 1986), neutrophils and eosinophils (Ukai et al., 1986; Frick et al., 1988; Styrt et al., 1988).
- Degranulation of mast cells (Trotter and Orr, 1973; Corrado et al., 1985) and increasing concentrations of biochemical mediators such as histamine, TAME-esterase and arachidonic metabolites (prostaglandin

D_2)(Goetzl, 1981; Naclerio et al., 1986; Brown et al., 1987; Linder et al., 1988). Degranulation of mast cells may cause hypersecretion (Konno et al., 1987b)

Late reaction

- Increasing concentrations of the mediators histamine and TAME-esterase but not the mediator prostaglandin D_2, suggesting that basophils are partly responsible for the late response (Naclerio et al., 1985; Norman, 1985).

Besides the immunological manifestations neurological manifestations have also been observed:

- Cholinergic hypersensitivity.
  (Kaliner et al., 1982; Corrado et al., 1986; Devillier et al., 1988)
- β-adrenoceptor hyposensitivity.
  (Kaliner et al., 1982; Shelhamer, 1983)
- Autoantibodies against β-adrenoceptors.
  (Venter et al., 1980; Kaliner et al., 1982)
- Histamine hypersensitivity.
  (Eccles, 1983; Corrada et al., 1986; Ukai et al., 1986)
- Sensory hypersensitivity.
  (Payan et al., 1984; Tønessen et al., 1988)

These data suggest a multifactorial pathogenesis of nasal hyperreactivity. Specific and non-specific nasal hyperreactivity may be due to (Mygind, 1982b):

1. an increase in the epithelial permeability.
   Mygind (1982b) proposed a hypothesis for the pathogenesis of immediate allergic rhinitis. Allergen exposure to the sensitised nasal mucosa increases the number of basophilic leucocytes and mast cells. Histamine, released from these cells, increases epithelial permeability and promotes allergen penetration and contact with other mast cells. Other authors suggested an increase of epithelial permeability by an increase in the number of eosinophils, which destruct the mucociliary apparatus (Frigas and Gleich, 1986; Frick et al., 1988). Histamine also increases vascular permeability and dilates blood vessels mainly via a direct effect on vascular H_1 and H_2 receptors. Itching, sneezing and hypersecretion are caused by

histamine effects on nervous H_1 receptors; hypersecretion being mediated via a parasympathetic reflex in the trigeminal and vidian nerve.

2. an increased sensitivity of the sensory nerves
   According to Payan et al. (1984), non-specific stimuli may increase the release of SP from the sensory nerves. The immediate effects of SP would be vasodilatation and activation of mast cells. The mast cell mediators could then amplify the initial inflammatory response.
3. a changed modulation of the afferent impulse.
   No data are available to support this hypothesis.
4. an increase in the number or sensitivity of receptors on effector cells, resulting in an imbalance of the autonomic nerve regulation.
   Cholinergic hyperresponsiveness and β-adrenergic hyporesponsiveness have been observed in allergic patients in comparison with controls (see neurological factors). Hyper- and hypo-responsiveness have been explained in terms of changes in neuroreceptor numbers in the lower airways of asthmatic patients (Szentivanyi, 1968; Barnes et al., 1980). Changes in the numbers of neuroreceptors in the nasal mucosa of allergic patients in comparison with chronic sinusitis patients have been reported (Ishibe et al., 1983).

1.5. Outline of the thesis.

The present study deals with the biochemical and autoradiographic analysis of neuroreceptors in the nasal mucosa in order to elucidate the supposed changes in the distribution pattern of the neuroreceptors in nasal hyperreactivity. Radioligand receptor binding and in vitro autoradiographic assays were developed for studying the biochemical characteristics, densities, sensitivities, subclasses, receptor-effector coupling and localization of neuroreceptors in the nasal mucosa. In the first instance, the rat was used as an experimental model, so that the restricted amount of human nasal mucosa was not a limiting factor.
The pharmacological characterization of muscarinic receptors in homogenates of rat nasal mucosa has partly been described (Rodrigues de Miranda et al., 1985; Klaassen et al., 1986b). In chapter 2, the muscarinic subclasses and the agonist binding to muscarinic receptors will be described. An in vitro autoradiographic assay was developed to localize the muscarinic receptors in the rat nasal mucosa and is described in chapter 3. The pharmacological characterization and localization of the β-adrenoceptors in rat nasal mucosa is described in chapter 4. The characterization of the α_1- and α_2-adrenoceptors in homogenates of rat nasal mucosa is described in chapter 5 and 6 respectively. Until now, it was not possible to locate these receptors.
Radioligand receptor binding and in vitro autoradiographic experiments were applied to human nasal mucosa. Biopsies were taken from patients with either an allergic or non-allergic rhinitis. The non-allergic patients were further subdivided into a control, a vasomotor rhinitis and a chronic sinusitis group (chapter 7). Biopsies were studied histologically to investigate the constituents of the biopsies used for the biochemical studies and for possible changes in nasal hyperreactivity (chapter 8). The pharmacological characterization and localization of the muscarinic and adrenergic receptors in the nasal mucosa of non-allergic and allergic patients will be described in chapter 9 and 10 respectively.

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MUSCARINIC ACETYLCHOLINE RECEPTORS IN HOMOGENATES OF RAT NASAL MUCOSA.

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2.1. Abstract

Muscarinic acetylcholine receptors have been demonstrated in homogenates of rat nasal mucosa (Rodrigues de Miranda et al., 1985; Klaassen et al., 1986). The relatively high Kd-value and the extraordinary homogeneous agonist binding sites reported in that study might be due to proteolysis. In this study the Kd-value of the ^{3}H-l-QNB binding was significantly lower and the competition of the agonist for the antagonist binding demonstrated 25±7% high affinity sites after the addition of protease inhibitors. In the presence of guanine nucleotides a monophasic inhibition curve was obtained.
The M_2 subtype selective antagonists AF-DX-116 and HHSiD inhibited ^{3}H-l-QNB with low and high affinity respectively, indicating that the M_2 receptors in rat nasal mucosa are of the M_2(gland) or M_3 subtype.

2.2. Introduction

Physiological and neurohistochemical experiments have demonstrated that the parasympathetic nervous system plays an essential role in the regulation of the vasomotor and secretory activities of the nasal mucosa (Grote et al., 1975; Anggård, 1977; Malm, 1983; Vecerina et al., 1983). Radioligand receptor binding studies have already demonstrated cholinergic muscarinic receptors in homogenates of rat nasal mucosa (Rodrigues de Miranda et al., 1985; Klaassen et al., 1986). Specific binding of ^{3}H-l-QNB (Quinuclidinylbenzilate), a cholinergic antagonist,

to rat nasal mucosa homogenates occurs to an homogeneous class of binding sites, with a dissociation constant of 0.06±0.02 nM and a receptor density of 8±2 pmol/g tissue (Klaassen et al, 1986). This binding is stereoselectively inhibited by benzetimide enantiomers, demonstrating the muscarinic character of the receptors. The agonists methylfurtrethonium and methacholine inhibit ^{3}H-l-QNB binding at high concentrations, pointing to the presence of an homogeneous population of low affinity agonist binding sites in 4 out of 6 experiments (Rodrigues de Miranda et al., 1985; Klaassen et al., 1986). This is rather exceptional because heterogeneity of agonist binding has been a predominant feature of muscarinic receptors in a variety of tissues (Birdsall, 1984; McKinney and Richelson, 1984; Nathanson, 1987).

Muscarinic receptors were subclassified on their selective profile of pirenzepine (Hammer et al., 1980; Birdsall, 1984). Pirenzepine displaces ^{3}H-l-QNB binding with low affinity ($5x10^{-6}$ M) in rat nasal mucosa, classifying the muscarinic binding sites as M_2 receptors (Rodrigues de Miranda et al., 1985; Klaassen et al., 1986). After the development of selective antagonists, it became clear that M_2 muscarinic receptors are not an homogeneous class. The subdivision of M_2 receptors in M_2(heart)(=$M_{2\alpha}$=M_{2A}) and M2(gland)(=$M_{2\beta}$=M_{2B}=M_3) subtypes has been demonstrated in functional and radioligand receptor binding studies by cardioselective antagonists such as AF-DX-116 and by glandselective antagonists such as Hexahydrosiladifenidol (HHSiD) (Mutschler and Lambrecht, 1984; Eglen and Whiting, 1986; Micheletti et al., 1987; Ladinsky et al., 1988). The pharmacological subdivision of M_2 receptors has been supported by molecular cloning studies (Kubo et al., 1986; Peralta et al., 1988).

In this study the muscarinic acetylcholine receptors were further characterized in homogenates of rat nasal mucosa with respect to the presence of M_2 muscarinic subclasses and their extraordinary agonist inhibition. In earlier studies inclusion of the protease inhibitors PMSF and EDTA during homogenization and incubation did not affect the binding parameters of the antagonist and agonist binding (Rodrigues de Miranda et al., 1985). In this study, the effect of the inclusion of other protease inhibitors, leupeptin and pepstatin, during homogenization and incubation

was investigated.

2.3. Materials and methods

^{3}H-l-QNB (Quinuclidinylbenzilate)(spec. act. 33.1 Ci/mmol) was purchased from New England Nuclear, Doorn, The Netherlands. AF-DX-116 was a gift from Dr. Karl Thomae GmbH; HHSiD and l-QNB were gifts from Dr. G. Lambrecht, Wolfgang Goethe University, Frankfurt, West-Germany. Methylfurtrethonium was synthesized in our laboratory according to known procedures. PMSF (phenylmethylsulfonylfluoride), Gpp(NH)p (5'-guanylylimidodiphosphate), leupeptin, pepstatin and HEPES were obtained from Sigma, Chemical Company, St. Louis, M.O., U.S.A. All other chemicals were of analytical grade.

The nasal mucosae from Wistar rats (about 200g body weight) were dissected, immediately frozen (acetone/CO_2 bath) and stored at -80^{o}C. The tissue was homogenized in icecold Tyrode buffer (pH 7.4) or HEPES-PI buffer (20 mM HEPES, 120 mM NaCl, 10 mM $MgCl_2$, 1 mM PMSF, 1 mM EDTA, 0.01 mM leupeptin and 0.01 mM pepstatin) with an Ultraturrax for 2x10 sec. The homogenate was centrifuged at 1000xg for 5 min. The supernatant was collected and centrifuged at 100,000xg for 1 hour (4^{o}C). The pellet was resuspended by Potter homogenization in Tyrode or HEPES-PI buffer.

Radioligand receptor binding studies were carried out in conical plastic centrifuge tubes, containing 40 μl ^{3}H-l-QNB, 40 μl Tyrode or HEPES buffer and 320 μl tissue homogenate (12.5 mg/ml Tyrode or HEPES-PI buffer). Non-specific binding was determined in the presence of 12 μM l-QNB. The homogenates were incubated for 90 min. at 37^{o}C under rotation (10 rpm) and centrifuged for 15 min. at 18,000xg. Aliquots (250 μl) of the supernatants were mixed with 10 ml of scintillation fluid (Aqua Luma, Lumac) for determining free concentrations of radioligand. The remaining supernatant was removed by suction. The tips of the centrifuge tubes were mixed with 10 ml Aqua Luma and the samples were counted in a liquid scintillation counter. The binding parameters were calculated by subjecting the data to a non-linear least squares curve fitting procedure using the Gauss Newton algorithm (Fletcher and Powell, 1963). Inhibition curves were analysed according to a one or two binding sites model.

2.4. Results

Specific ^{3}H-l-QNB binding to muscarinic receptors in homogenates of rat nasal mucosa in the presence of protease inhibitors (PMSF, EDTA, leupeptin, pepstatin) was saturable with a dissociation constant (Kd) of 0.04±0.02 nM and a receptor density (Bmax) of 9.0±2.6 pmol/g tissue (n=5) (fig. 2.1). The Kd-value of the ^{3}H-l-QNB binding with protease inhibitors was significantly lower than the Kd-value (0.06±0.02 nM) determined in the absence of protease inhibitors (Rodrigues de Miranda et al., 1985; Klaassen et al., 1986). All other experiments were performed in the presence of these inhibitors to be certain that maximal precaution was taken.

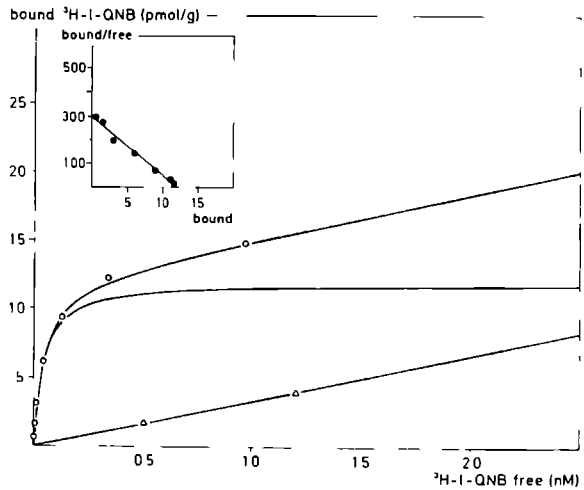

Fig. 2.1: Specific ^{3}H-l-QNB binding to homogenates of rat nasal mucosa in the presence of the protease inhibitors PMSF, EDTA, leupeptin and pepstatin. Total binding (O) and non-specific binding (△) were determined in the absence and presence of l-QNB. The line without points represents the specific ^{3}H-l-QNB binding. Inset: Scatchard plot of the specific part of the binding. Data shown are the means of triplicates from a representative experiment.

Inhibition of the full agonist methylfurtrethonium displayed a biphasic character (Ki_1=0.3±0.1 μM and Ki_2=7.0±0.5 μM) indicating the presence of 25±7% high affinity agonist binding sites (n=3)(fig. 2.2). In the presence of 10^{-4} M Gpp(NH)p the agonist inhibition curve was monophasic (Ki=8.9 μM), suggesting a conversion of the high into the low affinity agonist binding sites.

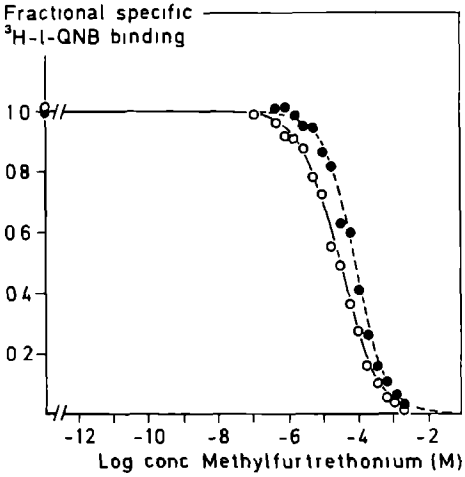

Fig. 2.2:
Inhibition of the specific ^{3}H-l-QNB binding to homogenates of rat nasal mucosa by the agonist methyfurtrethonium in absence (O) and presence (●) of 10^{-4} M Gpp(NH)p. The curve in the absence of Gpp(NH)p could significantly better fitted by a two binding sites model, whereas the curve in the presence of Gpp(NH)p could adequately be fitted by a one binding site model. Data shown are the means of triplicates.

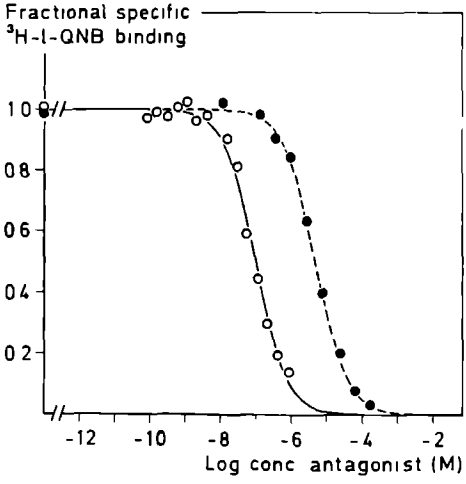

Fig. 2.3:
Inhibition of specific ^{3}H-l-QNB binding to homogenates of rat nasal mucosa by the antagonists AF-DX-116 (●) and HHSiD (O). The curves could adequately be fitted by a one binding site model. Data shown are the means of triplicates from a representative experiment.

The subtype selective antagonists AF-DX-116 (heart selective) and HHSiD (gland selective) were used to investigate muscarinic receptor subclasses in rat nasal mucosa. The inhibition curves of AF-DX-116 (Ki=0.98±0.13 μM; n=3), as well as HHSiD (Ki=0.025±0.003 μM; n=3) were monophasic, suggesting the presence of an homogeneous population of M_3 receptors in rat nasal mucosa (fig. 2.3).

2.5. Discussion

Specific ^{3}H-l-QNB binding to muscarinic receptors in homogenates of rat nasal mucosa has been described elsewhere (Rodrigues de Miranda et al., 1985; Klaassen et al., 1986). In that study the binding was of high affinity with Kd-value of 0.06±0.02 nM. This Kd-value appeared to be somewhat higher than described in other rat tissues (Watson et al., 1986; Batra, 1986; Gies et al., 1987). The agonist inhibition curve appeared to be monophasic in 4 out of 6 experiments. This is rather exceptional because heterogeneity of agonist binding has been a predominant feature of muscarinic receptors in a variety of tissues (Birdsall, 1984; Nathanson, 1987). Proteolysis of muscarinic receptors has been described in electrophoresis (Birdsall et al., 1979) and radioligand binding studies (Aronstam and Greenbaum, 1984; Batra, 1986). The higher Kd-value and the extraordinary homogeneous agonist binding sites demonstrated in earlier studies may be due to proteolysis and therefore PMSF and EDTA, serine and metalloprotease inhibitors respectively, were included during antagonist and agonist binding. However, inclusion of the protease inhibitors did not affect the binding parameters of antagonist and agonist binding (Rodrigues de Miranda et al., 1985).
In this study pepstatin and leupeptin, inhibitors of carboxyl and thiolproteases respectively, were included in addition to PMSF and EDTA during homogenization and incubation. The Kd-value (0.04±0.02 nM) of the ^{3}H-l-QNB binding to homogenates of rat nasal mucosa was significantly lower in the presence of the four protease inhibitors in comparison with the Kd-values determined in the presence of PMSF and EDTA alone. The

present results show that in homogenates of rat nasal mucosa the agonist methylfurtrethonium binds to high (25±7%) and low affinity agonist binding sites. The Ki-values were in agreement with those described in bovine (Beld et al., 1980) and human tracheal smooth muscle (van Koppen et al., 1985). It has been accepted that the high affinity complex consists of agonist, receptor and guaninenucleotide binding protein (Birdsall, 1984; Nathanson, 1987). In the presence of 10^{-4} M Gpp(NH)p a monophasic inhibition curve was observed. This complete conversion of the high into the low affinity agonist binding sites has been demonstrated in other tissues (Birdsall, 1984; Nathanson, 1987), but incomplete conversion has also been observed (Vanderheijden et al., 1987; Rinner et al., 1988).

The M_2(heart) and M_2(gland) selective antagonists AF-DX-116 and HHSiD inversely inhibited the ^{3}H-l-QNB binding in membranes of rat nasal mucosa. AF-DX-116 showed only low affinity, whereas HHSiD showed only high affinity for the ^{3}H-l-QNB binding sites, suggesting an homogeneous population of M_2(gland) subtypes, also described as M_3 receptors, in rat nasal mucosa. The Ki-values of AF-DX-116 inhibition were in agreement with those determined in rat submandibular glands (Martos et al., 1987) and in rat pancreas (Waelbroeck et al., 1987); the Ki-values of HHSiD inhibition were in agreement with those reported in rat submaxillary glands (Baudière et al., 1987) and in rat pancreas (Waelbroeck et al., 1987). The presence of M_2(gland) subtypes in rat nasal mucosa is supported by in vivo and in vitro autoradiography (Klaassen et al., 1984; van Megen et al., 1988). The autoradiograms demonstrated specific ^{3}H-l-QNB labelling of muscarinic receptors in rat nasal glands.

In further experiments, agonist and antagonist binding will be performed in the presence of protease inhibitors in human nasal mucosa to study the supposed changes in the distribution pattern of receptors under pathological conditions such as nasal hyperreactivity.

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AUTORADIOGRAPHIC ANALYSIS OF MUSCARINIC RECEPTORS IN RAT NASAL GLANDS.

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3.1. Abstract

An in vitro method was developed for the biochemical and autoradiographic demonstration of low muscarinic receptor densities in peripheral tissue. Histological criteria point clearly to the necessity for fixation to preserve tissue quality. ^{3}H-l-Quinuclidinylbenzilate bound specifically to a homogeneous class of binding sites in 0.5% glutardialdehyde-fixed cryostat sections (10 μm) of rat nasal glands with high affinity (Kd=0.47±0.06 nM) and with a receptor density (Bmax) of 41±1 fmol/mg protein. This binding was linearly dependent on the thickness of the sections. Kinetic experiments resulted in a Kd-value of 0.19 nM. Binding was stereoselctively inhibited by benzetimide enatiomers. Autoradiograms, generated after incubation with 0.6 nM ^{3}H-l-Quinuclidinylbenzilate and dipping in nuclear K2 emulsion, showed specific labelling of glandular acini and excretory ducts. These in vitro observations provide conclusive evidence for the presence of acetylcholine receptors in the nasal glands of the rat.

3.2. Introduction

The parasympathetic nervous system plays an essential role in the regulation of the vasomotor and secretory activities of the nasal mucosa.

Physiological experiments in animals have demonstrated the presence of acetylcholine receptors in nasal vessels and glands. Metacholine causes a vasodilatation, which can be blocked by atropine (Malm, 1983). Electrical stimulation of parasympathetic nerves in the nasal glands provokes secretion which can be inhibited by atropine (Eccles and Wilson, 1973; Anggård, 1974; 1977). Neurohistochemical investigations also point to the existence of a well developed parasympathetic nervous system in the respiratory part of the nasal mucosa (Ishii and Toriyama, 1972; Grote et al., 1975; Vecerina et al., 1983). The innervation of the nasal glands appears to consist of acetylcholinesterase-containing fibres. The vascular structures, especially the sinusoids, also show a very dense network of these fibres.

Radioligand receptor binding assays could provide more quantitative data on the density and biochemical characteristics of the receptors involved. Muscarinic acetylcholine receptors have been described in the nasal mucosa of the rat (Rodrigues de Miranda et al., 1985), guinea pig (Ishibe et al., 1985; Konno et al., 1985) and man (Ishibe et al., 1983) with the help of the anatagonist ^{3}H-l-QNB (^{3}H-l-Quinuclidinylbenzilate). These investigations were performed on homogenates of the nasal mucosa and do not give any information about the localization of the receptors. In vitro autoradiography seems a suitable technique for the localization of receptors (Young and Kuhar, 1979; Wamsley, 1983; Nonaka and Moroji, 1984; Palacios, 1984).

Precautions have been taken to minimize diffusion of the ligand from the receptor when autoradiographic techniques are applied. This would exclude the autoradiographic dipping technique (Wamsley, 1983). High affinity radioligands are now available and the technique of dipping the slides into liquid emulsion seems to be applicable. The present study was designed in an attempt to develop an in vitro autoradiographic technique for the localization of muscarinic receptors in rat nasal glands. In principle, three methods of covering the tissue sections with a photographic sensitive layer are compared. Furthermore, it is necessary that this in vitro technique should also be applicable for the pharmacological identification of the receptors involved.

3.3. Materials and methods

Tissue preparation

Wistar rats (about 200 g body weight) were used throughout this investigation. After decapitation, the nasal glands were carefully dissected and rapidly frozen using isopentane cooled in liquid nitrogen. Cryostat sections were thaw-mounted on glass slides coated with gelatin/chrome-alum. The sections were air dried and stored at -80°C. Before incubation the sections were dried again at room temperature and fixed at 4°C in 0.5 or 2% glutardialdehyde in Tyrode buffer for 30 or 45 min dependent on the thickness of the section. Subsequently, the sections were rinsed in buffer at 4°C for 30 min and further processed for either radioreceptor assays or autoradiography. Alternatively, instead of this fixation, inhibitors of proteolysis (1 mM PMSF, 5 mM EDTA, 0.01 mM Leupeptin, 0.01 mM Pepstatin) were included during the incubation procedure as described below.

Several non-mounted parallel sections were collected for protein determination according to Lowry et al. (1951) with bovine serum albumin as a standard. Protein determination was performed after solubilization of the sections in 0.1 N NaOH at 100°C (boiling water-bath) for 15 min. Parallel sections, taken at various stages of the incubation procedures were stained with methylgreen pyronin (1%) for histological verification of tissue quality.

Radioreceptor assay on cryostat sections.

The tissue sections were overlayed with 150 µl ^{3}H-l-QNB (specific activity 33.1 Ci/mmol, NEN, Doorn, The Netherlands) in Tyrode buffer at various concentrations ranging from 0.04-8.0 nM. Subsequently, the sections were incubated at 37°C for 90 min in a humid atmosphere. Non-specific binding was measured in parallel incubations in the presence of 12 µM L-QNB (gift from Dr. G. Lambrecht, Johan Wolfgang Goethe Univerität, Frankfurt/m, West-Germany) under the same conditions. After the incubation 75 µl of the incubation buffer was mixed with 10 ml Aqua Luma (Packard) to determine the concentration of radioligand. The remaining incubation buffer was removed by rinsing the sections in fresh

buffer. The slides with the sections were then placed in counting vials, containing 1 ml Soulene-350 (Packard). The vials were warmed to 37°C overnight. Instagel/0.5 N HCl (9:1) 18 ml, was added and the samples were then counted in a liquid scintillation counter (1215 Rackbeta, LKB). The binding parameters were calculated by subjecting the data to a non-linear least squares curve fitting procedure with the Gauss-Newton algorithm. Identical results were obtained with this droplet incubation method and with the incubation of sections on slides in jars (Young and Kuhar, 1979).

Autoradiography.

Following the method described above, 10 μm sections of rat nasal glands were incubated with 0.06 nM ^{3}H-1-QNB at 37°C. Adjacent sections were incubated with 12 μM L-QNB in addition to ^{3}H-1-QNB to determine the non-specific binding. After incubation, the sections were rinsed in Tyrode buffer (room temperature) then briefly in distilled water to remove the buffer salts. The specimens were coated with 0.5% gelatin/chrome-alum. Three methods were used for covering the slides with photographic sensitive layer and further processing: (a) The slides were air-dried and dipped in liquid emulsion (nuclear K2 emulsion, Ilford) diluted with distilled water (1:1). After an exposure time varying between 14 days and 3 months at 4°C the autoradiograms were developed in amidol (18°C, 8 min.), rinsed in distilled water, fixed in sodium thiosulphate (30%) at 18°C for 1-3 min, thoroughly washed in distilled water (15 min) and stained with 1% methylgreen pyronin. (b) Several sections were covered with Kodak AR-10 stripping film swollen in distilled water (room temperature) for 2-3 min. After an exposure time ranging from 1-3 months at 4°C, the autoradiograms were developed in Kodak D19 developer, rinsed briefly in clean water (18°C) and fixed in a solution prepared according to Kodak formula F-24. The slides were then extensively washed in deionized water, air-dried and stained with 1% methylgreen pyronin. (c) Autoradiograms were generated by apposition to emulsion (nuclear K2 emulsion, Ilford)-coated coverslips according to the method previously described by Young and Kuhar (1979). After an exposure time ranging from 1-3 months, development of the autoradiograms was carried out according

to method (a) after the coverslips had been gently bent away from the tissue sections.

3.4. Results

Histological tissue integrity and characteristics of the acetylcholine receptor involved were examined before autoradiographic experiments were performed.

Histological experiments.

The nasal glands of the rat are of the tubulo-acinar type. Acinar cells and striated excretory ducts were observed, all embedded in connective tissue.

Methylgreen pyronin stains the nuclei green-blue and the acinar cytoplasm purple-red. There was no essential difference in staining reaction with methylgreen pyronin whether the tissue sections were fixed in 0.5% or in 2% glutardialdehyde. Omitting fixation in the absence or presence of inhibitors of proteolysis during incubation resulted in a loss of staining reaction, many cytoplasmatic vacuoles and a diffuse appearance of the nuclei.

Biochemical experiments.

Tissue sections of the nasal glands fixed in 0.5% glutardialdehyde were used throughout these experiments since lack of fixation led to a marked loss of tissue integrity. Figure 3.1 represents a typical experiment on the binding of ^{3}H-l-QNB to 10 μm cryostat sections of rat nasal glands. The specific part of the binding was saturable with a receptor density (Bmax) of 39 fmol/mg protein and a dissociation constant (Kd) of 0.43 nM. The Scatchard plot (inset) points to the presence of a homogeneous class of binding sites. The non-specific binding was relatively low. Both Kd and Bmax varied slightly between experiments and a Kd of 0.47±0.06 nM and a Bmax of 41±1 fmol/mg protein was calculated from the mean of three experiments. There was no difference in binding parameters between ^{3}H-l-QNB binding in 10 or 20 μm cryostat sections. The binding of ^{3}H-l-QNB was linearly dependent on the thickness of the sections.

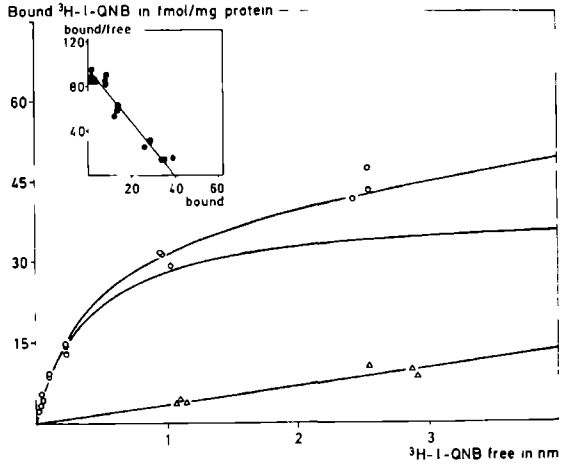

Fig. 3.1: Binding of ^{3}H-1-QNB to 0.5 % glutardialdehyde fixed cryostat sections (10 µm) of rat nasal glands at 37°C. Total binding (O) and non-specific binding (Δ) which was determined in the presence of 12 µM unlabelled QNB. The line without points represents the specific ^{3}H-1-QNB binding. The dissociation constant was 0.43 nM and the receptor density 39 fmol/mg protein. Inset: Scatchard plot of the specific part of the binding.

Kinetic experiments, in which bound radioligand was determined as a function of time, indicated that equilibrium was reached after 90 min at a ligand concentration of 0.4 nM (fig. 3.2). Dissociation of the ligand-receptor complex proceeded slowly with a half-life of approximately 90 min. The association rate constant was $4.1 \times 10^{7} M^{-1}.min^{-1}$. The dissociation rate constant was 0.0076 min^{-1}. Consequently, the calculated kinetic Kd was 0.19 nM. There was no difference in the kinetics of ^{3}H-1-QNB binding between to 10 and 20 µm sections.

The binding of ^{3}H-1-QNB (1.3 nM) to muscarinic receptors in sections of rat nasal glands was inhibited stereoselectively by stereoisomers of the antagonist benzetimide. Dexetimide was 1500 times as effective in displacing ^{3}H-1-QNB from its binding sites as levetimide ($Ki=8.3 \times 10^{-9}$ and $Ki=1.2 \times 10^{-5}$ M, respectively).

In all these binding studies incubations were terminated by rinses in

fresh buffer. It appears from fig. 3.3 that rinses of 3x4 min reduced the non-specific binding of the radioligand to the sections and resulted in an optimal ratio of specific to non-specific binding. Rinses of 3x10 min were sufficient to obtain the same ratio of specific to non-specific binding in 20 µm sections.

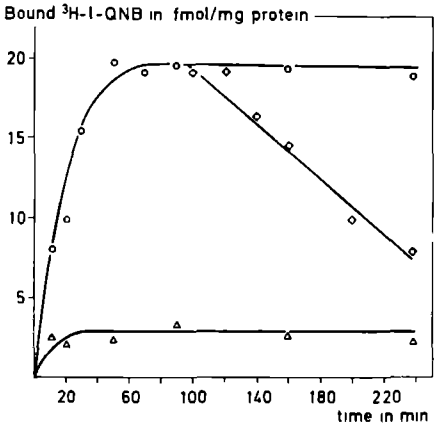

Fig. 3.2:
Time dependent binding of ^{3}H-1-QNB (O) (0.4 nM) to 0.5% glutardialdehyde-fixed cryostat sections (10 µm) of rat nasal glands. The non-specific (△) binding was relatively low. Dissociation of the radioligand receptor binding complex was initiated by the addition of an excess of unlabelled ligand (12 µM) (◇).

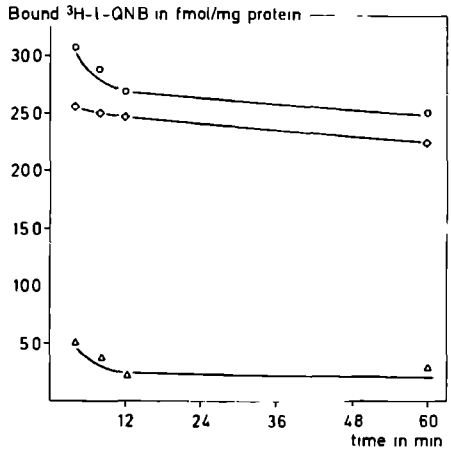

Fig. 3.3:
The influence of the rinsing time on the dissociation of the radioligand receptor complex. Fixed tissue sections were incubated with 0.9 nM ^{3}H-1-QNB for 90 min. at 37°C. The slides were then rinsed in fresh buffer for times varying from 4 to 60 min. Total binding (O), specific binding (◇), non-specific binding (△).

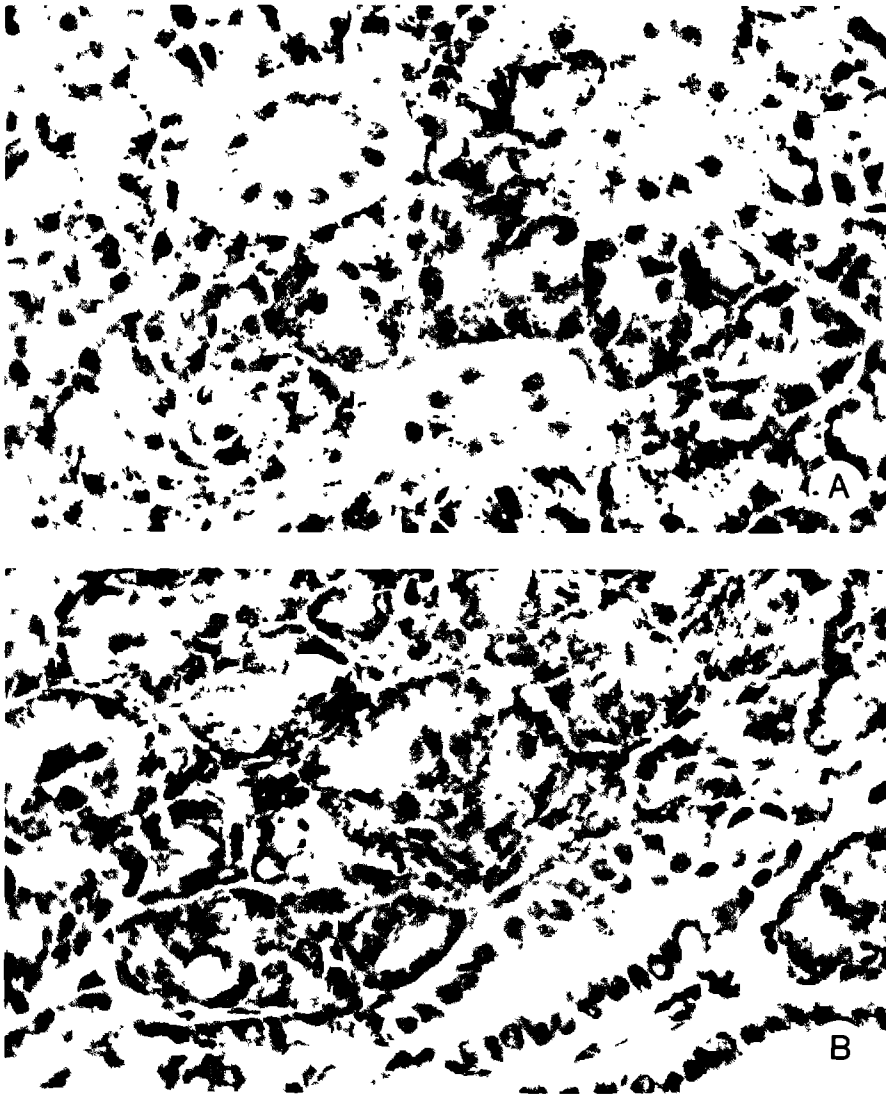

Fig. 3.4:
Autoradiograms of 0.5% glutardialdehyde-fixed cryostat sections (10 µm) of rat nasal glands after incubation with 0.6 nM ^{3}H-1-QNB (A) or with an excess of 1-QNB in addition to the radioligand (B) followed by dipping in liquid nuclear emulsion. The autoradiograms show specific labelling of the glandular acini and the excretory ducts (x800). Staining with methylgreen pyronin (1%).

Autoradiographic experiments.

After 3 months exposure the autoradiographs showed labelling of the glandular acini with ^{3}H-1-QNB (fig. 3.4A). The excretory ducts also showed slight labelling, whereas the connective tissue was devoid of silver grains. Autoradiographs made from sections incubated with an excess of unlabelled ligand in addition to 0.6 nM ^{3}H-1-QNB, showed only a very faint homogeneous labelling of the glandular tissue (fig. 3.4B).

These autoradiograms were generated by means of the dip-method. The most relevant disadvantage of the other two autoradiographic techniques was the appearance of high background levels, making difficult the detection of small amounts of specific binding.

3.5. Discussion

Most reports on the autoradiographic localization of different receptors deal with the high receptor densities found in the brain (Young and Kuhar, 1979; Wamsley, 1983; Palacios, 1984) and only a few deal with lower receptor concentrations (Barnes et al., 1983; Summers et al., 1984). The droplet incubation using a small amount of radioligand in combination with the dip method offers the possibility of characterizing and localizing peripheral muscarinic receptors at low receptor concentrations.

A well preserved histology is necessary for accurate localization of the silver grains. The commonly used fixatives such as glutardialdehyde are generally recommended because they do not interfere with the autoradiographic technique (Pearse, 1980). Fixation with glutardialdehyde appeared to be indispensable because the absence of fixation resulted in deformation of the incubated glandular tissue. Unfortunately, this implies that it is impossible to perform control experiments to check the influence of the fixatives on ligand receptor binding in cryostat sections. Young and Kuhar (1979), suggested on the basis of in vivo experiments that low concentrations of formaldehyde do not result in a loss of opioid receptors in rat brain. The concentration of glutardialdehyde (0.5%) used in the present study was also low since no difference in histology of the nasal glands was observed after fixation

with either 0.5 or 2% glutardialdehyde.

The receptor binding studies showed that ^{3}H-l-QNB binds to a homogeneous class of muscarinic receptors in both 10 and 20 µm sections of rat nasal glands. The binding was saturable and of high affinity. The muscarinic nature of the receptors was confirmed by the inhibition of ^{3}H-l-QNB binding by the enantiomers of benzetimide. The receptor density (41±1 fmol/mg protein) in rat nasal glands was low in contrast with the density of 2000 fmol/mg protein in rat striatum cryostat sections (10 µm)(Nonaka and Moroji, 1984). Comparing our findings with data from studies on homogenates reveals that the receptor density found in the present study agrees well with the density of nasal glandular homogenates (55 fmol/mg protein)(Klaassen et al., in press) and is lower than the density in the submandibular gland (214 fmol/mg protein)(Costa and Murphy, 1985). The Kd-value (0.47±0.06 nM) agreed well with the value found for ^{3}H-l-QNB binding in cryostat sections of the rat striatum (0.36 nM)(Nonaka and Moroji, 1984), but contrasts with the value found in ferret lung (0.06 nM)(Barnes et al., 1983). Nonaka and Moroji (1984) demonstrated a difference in Kd-values between 10 and 20 µm cryostat sections and suggested a dependence of the Kd value upon receptor density. This suggestion is not supported by the binding and kinetic experiments of the present study. There are slight differences between values for ^{3}H-l-QNB binding to cryostat sections and those for homogenates of rat nasal mucosa (Kd=0.06 nM)(Rodrigues de Miranda et al., 1985) and of rat nasal glands (Kd=0.11 nM)(Klaassen et al., 1987). Such discrepancies have already been reported (Gilbert et al., 1979; Nonaka and Moroji, 1984), but Barnes et al. (1983) found identical Kd-values for ^{3}H-l-QNB binding to cryostat sections and homogenates of the ferret lung. If methodological aspects are left out, these discrepancies should be due to the inability of the radioligand to penetrate the sections (Wamsley et al., 1981; Nonaka and Moroji, 1984). However, the binding of ^{3}H-l-QNB appeared to be linearly dependent on the thickness of the sections, whereas the Kd-value was independent of the thickness of the sections. Alternatively, the affinity of the ligand for the receptor in more or less intact membranes of cryostat sections could be different from that in homogenized membrane fragments.

After an exposure time of 3 months, the autoradiographs of 10 μm sections showed specific labelling of the acini with ^{3}H-l-QNB. This observation provides evidence for the presence of muscarinic acetylcholine receptors in rat nasal glands. The findings are also in agreement with in vivo autoradiographic results (Klaassen et al., 1984), and with results of histochemical studies on the cholinergic innervation of the nasal mucosa (Grote et al., 1975) and of physiological experiments (Klaassen and Kuijpers, 1986). In addition to the findings in previous studies, the autoradiographs also suggest a slight specific labelling of the striated excretory ducts. The striated ducts of the salivary gland control the ion and water content of the secretions (Schneyer et al., 1972), and the striated ducts of the nasal glands could have a similar function (Phipps, 1981). It can be deduced from the present data that this function is under parasympathetic control.

The best autoradiographic results were obtained from the dipping technique. In contrast to this method, the slides covered with Kodak stripping film showed increased background levels after longer exposure times. The increased background levels interfered with the detection of small amounts of silver grains above the section. Floating the stripping film on a sugar solution of bromide (10 mg/ml) may suppress the high background levels (O'Callaghan et al., 1969). The Ilford K2 emulsion-coated coverslip method also showed such increased background levels. Rogers (1973) has already reported that pressure causes latent images of such films. A disadvantage of the dipping technique, however, is that it gives no reliable quantitative data on grain density because dipping does not guarantee a uniform thickness of the emulsion layer (Rogers, 1973). Many other problems can also be encountered in quantitative receptor mapping (Rogers, 1973; Kuhar and Unnerstal, 1985; Kuhar, 1985).

The technique now presented, that involves incubation of the sections with a small amount of ^{3}H-l-QNB and covering the slides with photographic emulsion, seems a suitable method for the localization of low receptor densities in human nasal mucosa and for studying the presumed changes in the distribution pattern of receptors under pathological conditions such as nasal hyperreactivity.

Acknowledgement

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BIOCHEMICAL AND AUTORADIOGRAPHIC ANALYSIS OF β-ADRENOCEPTORS IN RAT NASAL MUCOSA

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4.1. Abstract

The specific binding of ^{125}I-(-)-cyanopindolol to homogenates and cryostat sections of rat nasal mucosa was saturable, stereoselective and of high affinity (Kd=5.0±0.4 pM, Bmax=204±12 fmol/mg protein and Kd=7.2±0.7 pM; Bmax=15±1 fmol/mg protein respectively). The subtype selective antagonists IK$_{203-030}$ and ICI$_{118,551}$ inhibited specific ^{125}I-(-)-CYP binding, in both homogenates and cryostat sections, according to a two binding sites model indicating the presence of β_1- and β_2-adrenoceptors. Competition of isoprenaline for the antagonist binding to homogenates demonstrated 30% high affinity agonist binding sites. In the presence of guanine nucleotides a steepening of the curve was observed.
In vitro labelling of cryostat sections of rat nasal mucosa was combined with autoradiography. The autoradiographs, generated after incubation with 20 pM ^{125}I-(-)-CYP, showed specific labelling of the epithelium and glandular excretory ducts. It appeared from autoradiographs generated with subtype selective antagonists in addition to the radioligand that β_1- and β_2-adrenoceptors were present in both structures.

4.2. Introduction

Physiological and morphological experiments have shown that especially the nasal vascular structures are innervated by sympathetic (adrenergic) nerves (Anggård and Densert, 1974; Grote et al., 1975; Malm, 1977; Vecerina et al., 1983). Stimulation of the sympathetic nerves results in a release of noradrenaline, which activates α- and β-adrenoceptors. Although it is well established that α-adrenoceptors predominate in nasal vessels, the opinion of the presence of β-adrenoceptors is controversial. Hall and Jackson (1968) could not demonstrate β-adrenoceptors in the nasal blood vessels of the dog. On the other hand, these receptors have been demonstrated in nasal resistance or capacitance vessels in the cat and the dog (Malm, 1977; Hiley et al., 1978). No evidence for adrenergic innervation of the nasal glands has been obtained. However, some physiological experiments suggested an influence of adrenergic agonists on the secretory activities of the nasal mucosa (Phipps, 1981; Malm et al., 1983).

Clinical pharmacological investigations point to the anti-allergic effect of β-adrenergic agonists by inhibiting histamine release from mediator cells, suggesting a role of these receptors in mediator release (Kaliner and Austen, 1975; Svensson et al., 1980). Quantitative data on biochemical characteristics and localization of the β-adrenoceptors are necessary to get more insight in the physiological role of these receptors in the nasal mucosa. In this study radioligand binding techniques were applied for the biochemical characterization of β-adrenoceptors in homogenates and in cryostat sections of rat nasal mucosa, using the antagonist ^{125}I-(-)-cyanopindolol (^{125}I-(-)-CYP). The β-adrenoceptors were characterized in regard to affinity, receptor density, stereoselectivity, subclassification in β_1- and β_2-adrenoceptors and the inhibitory pattern of the antagonist binding by the agonist isoprenaline. The in vitro labelling of the cryostat sections was combined with autoradiography for the localization of β-adrenoceptors in rat nasal mucosa.

4.3. Materials and methods

^{125}I-cyanopindolol [^{125}I-(-)-CYP](spec. act. 2200 Ci/mmol) was purchased from Amersham International, England. $ICI_{118,551}$, (-)- and (±)-propranolol were gifts from ICI Ltd, Alderly Park, England, (±)-isoprenaline and $LK_{203-030}$ from Boehringer Ingelheim, FRB and Sandoz, Basel, Switzerland respectively. (+)-propranolol was purchased from Sigma Chemical Company, St. Louis, MO, USA.

Radioligand receptor binding assay for homogenates

The nasal mucosae from Wistar rats (about 200 g body weights) were dissected, immediately frozen (acetone/CO_2 bath) and stored at -80^{0}C. The tissue was homogenized in icecold Tyrode buffer (pH 7.4) with an Ultraturrax for 2x10 sec. The homogenate was centrifuged at 1000 g for 5 min The supernatant was collected and centrifuged at 100,000 g for 1 hr (4^{0}C). The pellet was resuspended by Potter homogenization in buffer. Radioligand receptor binding studies were carried out in glass tubes containing 40 µl ^{125}I-(-)-CYP, 40 µl Tyrode buffer with 0.1% BSA and 320 µl tissue homogenate corresponding with a final concentration of 2 mg/ml tissue. Non-specific binding was determined in the presence of 2 µM (±)-propranolol. The homogenates were incubated for 2 hrs at 37^{0}C.
Incubations were terminated by rapid dilution with 2x2 ml ice cold buffer and filtration through Whatman GF/C filters under vacuum, followed by 3x5 ml rinses of the filter with ice cold buffer. Radioactivity collected on each filter was measured in a gammacounter (NE1612, NEN) at 78% counting efficiency. In order to obtain free radioligand concentration total concentration was corrected for bound concentration. The binding parameters were calculated by subjecting the data to a non-linear least square curve fitting procedure using the Gauss-Newton algorithm (Fletcher and Powell, 1963). Inhibition curves were analysed according to a one or two binding sites model. The half life time ($t_{½}$) for equilibration was calculated by the equation (Graafsma et al., 1988):

$$t_{½} = \frac{2.3}{c.k1} \frac{\log(A+3C)}{(A+C)}$$ with $A=Ro+Do+Kd$, $B=Ro.Do$ and $C=(A^2-4B)^{½}$

Do=initial ligand concentration

Ro=total receptor concentration

Radioreceptor binding assay for cryostat sections.

The nasal mucosae from Wistar rats were carefully dissected and rapidly frozen using isopentane cooled in liquid nitrogen. Cryostat sections (10 μm) were thaw-mounted on 1% gelatin/chrome-alum coated glass slides. Several non-mounted parallel sections were solubilized in 0.1 N NaOH at 100°C (boiling-waterbath for 15 min) for protein determination according to Lowry (1951) with Bovine Serum Albumine (BSA) as a standard. The sections were dried and stored at -80°C. Before the incubation, sections were dried again at room temperature (r.t.) and fixed in 0.5% glutardialdehyde in Tyrode buffer for 30 min (4°C) followed by rinsing in buffer (2x15 min; 4°C). The tissue sections were dried and overlayed with 150 μl ^{125}I-(-)-CYP in Tyrode buffer (with 0.01% BSA) at various concentrations radioligand. Subsequently, the sections were incubated at 37°C for 2 hrs in an humid atmosphere. For the measurements of the non-specific binding, parallel incubations were performed in the presence of 2 μM (±)-propranolol at the same conditions. After the incubation, 75 μl of the incubation buffer was counted to determine the free concentration radioligand, the remaining incubation buffer was removed by rinsing the sections in fresh buffer (r.t.) during 4x15 min The slides were broken, transferred to vials and the radioactivity in the sections was counted by gamma counting (NE1612 gammacounter, NEN). The binding parameters were calculated as described above.

In vitro autoradiography.

Incubations on cryostat sections were performed as described above using 0.02 nM ^{125}I-(-)-CYP. After washing the slides in buffer (r.t.), they were rinsed in distilled water (r.t.) to remove salts. The specimens were coated with 0.5% gelatin/chrome alum, air dried and dipped in liquid emulsion (Nuclear K_2 emulsion, Ilford) diluted with distilled water (1:1). After 2 months exposure (4°C) the autoradiographs were developed in amidol (18°C, 8 min), rinsed in distilled water, fixed in sodium thiosulphate (30%) at 18°C for 1-3 min, thoroughly washed in distilled water (15 min) and stained with 1% methylgreen pyronin.

Characterization of ^{125}I-(-)-CYP binding to homogenates of rat nasal mucosa.

Specific binding of ^{125}I-(-)-CYP to membranes of rat nasal mucosa was saturable and of high affinity (fig. 4.1). The Scatchard plot (inset) points to the presence of a homogeneous class of binding sites. Analysis of the data demonstrated a mean dissociation constant (Kd) of 5.0 ± 0.4 pM and a receptor density (Bmax)of 5.1 ± 0.3 pmol/g tissue corresponding with 204 ± 12 fmol/mg protein (n=10).

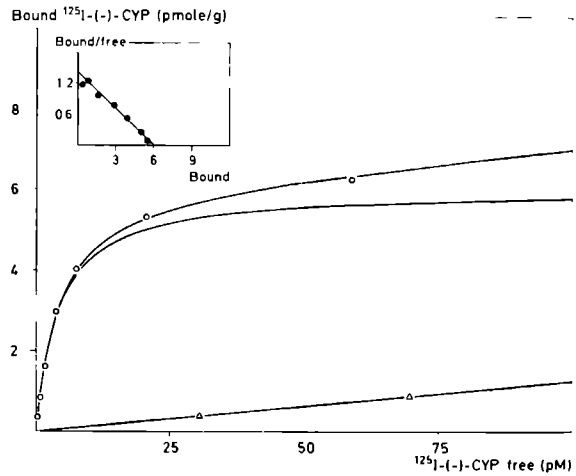

Fig. 4.1: Specific ^{125}I-(-)-CYP binding to homogenates of rat nasal mucosa. Total binding (0) and non-specific binding (△) were determined in the absence and presence of 2 μM (±)-propranolol. The line without points represents the specific ^{125}I-(-)-CYP binding. Inset: Scatchard plot of the specific part of the binding. Data shown are the means of triplicates from a representative experiment.

^{125}I-(-)-CYP binding to rat nasal mucosa was linearly dependent on tissue concentration between 0.5 and 20 mg/ml at a free ligand

concentration of 0.02 nM. The time dependent association and dissociation from β-adrenergic receptors in rat nasal mucosa was studied in the presence of 3 pM ^{125}I-(-)-CYP (fig. 4.2). Binding of ^{125}I-(-)-CYP at 37^{0}C reached equilibrium at 120 min The association rate constants (k_1), determined in two experiments, were 1.80 and 1.57 nM^{-1} min^{-1}. The dissociation rate constants (k_{-1}) were 0.0022 and 0.0024 min^{-1}. Consequently, the calculated Kd-values ($Kd=k_{-1}/k_1$) were 1.2 pM and 1.5 pM respectively. The halflife times of reaching equilibrium were 35 and 39 min respectively.

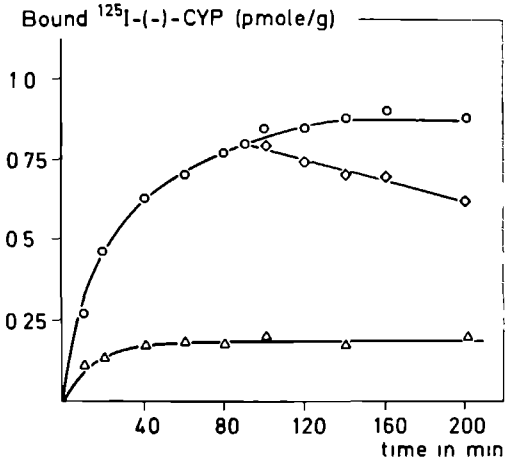

Fig. 4.2:
Time course of association and dissociation of ^{125}I-(-)-CYP binding to homogenates of rat nasal mucosa. Incubations were performed with 3 pM ^{125}I-(-)-CYP at 37^{0}C. Dissociation was induced by addition of 2 μM (±)-propranolol after 90 min incubation. Total binding (o), non-specific binding (△) and dissociation (◇). Data shown are the means of triplicates.

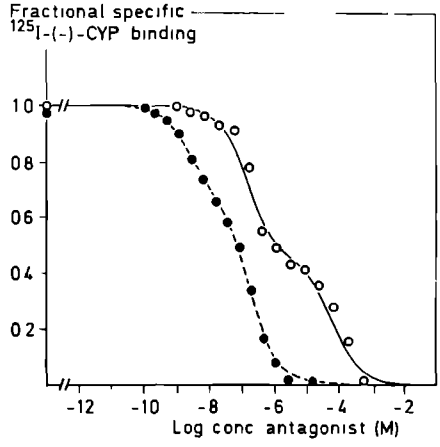

Fig. 4.3:
Inhibition of specific ^{125}I-(-)-CYP binding by the antagonists $LK_{203-030}$ (0) and $ICI_{118,551}$ (●). Homogenates of nasal mucosa were incubated at 37^{0}C for 120 min at 16 pM ^{125}I-(-)-CYP in the presence or absence of competing agents. Data shown are the means of triplicates from a representative experiment.

Table 4.1: Inhibition constants of β-adrenergic antagonists for ^{125}I-(-)-CYP (16 pM) binding to homogenates and cryostat sections of rat nasal mucosa.

Antagonist	Ki_1 (nM)	Ki_2 (μM)	% β_1	n
Homogenates				
(-)-propranolol	0.75/ 0.76	-		n=2
(+)-propranolol	120 / 90	-		n=2
$LK_{203-030}$	42 ± 11	17.5±3.9	50±4	n=3
$ICI_{118,551}$	0.9 ± 0.3	0.01±0.02	63±4	n=3
Cryostat sections				
(-)-propranolol	0.49/ 0.78	-		n=2
(+)-propranolol	260 / 320	-		n=2
$LK_{203-030}$	24 ± 1	48±14	43±11	n=3
$ICI_{118,551}$	7 ± 4	0.26±0.10	45±5	n=3

The binding of ^{125}I-(-)-CYP (16 pM) to β-adrenoceptors in homogenates of rat nasal mucosa was stereoselectively inhibited by stereoisomers of the antagonist propranolol. (-)-propranolol appeared to be a 140 fold more potent than (+)-propranolol in competing for ^{125}I-(-)-CYP binding sites (table 4.1). Competition curves could adequately be fitted with a one binding site model.

The subtype selective β-adrenergic antagonists $LK_{203-030}$ (β_1-selective) and $ICI_{118,551}$ (β_2-selective) were used to define the relative proportions of β_1 and β_2-adrenoceptors in rat nasal mucosa homogenates (table 4.1/fig. 4.3). Both competition curves were shallow and could be fitted with a two binding site model. Inhibition of ^{125}I-(-)-CYP by $LK_{203-030}$ as well as $ICI_{118,551}$ demonstrated the presence of a heterogeneous β-adrenoceptor population in rat nasal mucosa, $LK_{203-030}$ detected 50±4% β_1-adrenoceptors and $ICI_{118,551}$ detected 63±4% β_1-adrenoceptors.

Inhibition curves of the full agonist isoprenaline displayed a biphasic character indicating the presence of high (30±3%) and low affinity agonist binding sites in the rat nasal mucosa (table 4.2). In the presence of 5.10^{-4} M Gpp(NH)p a steepening of the isoprenaline inhibition curve was observed. However, no complete conversion of high affinity binding sites into low affinity binding sites could be obtained.

Table 4.2: Inhibition constants of the β-adrenergic agonist isoprenaline for ^{125}I-(-)-CYP binding to homogenates of rat nasal mucosa.

	Ki_1 (μM)	Ki_2 (μM)	% RH	n
- Gpp(NH)p	0.027±0.006	0.60±0.07	30±3	3
+ Gpp(NH)p	0.14 ±0.06	1.3 ±0.4	50±18*	3

* RH: A different set of affinity states with a lower agonist affinity than the high affinity state determined without Gpp(NH)p.

Characterization of ^{125}I-(-)-CYP binding to sections of rat nasal mucosa.

The specific binding of ^{125}I-(-)-CYP to 10 μm cryostat sections of rat nasal mucosa is saturable (fig. 4.4). The Scatchard plot (inset) points to the presence of a homogeneous class of binding sites with high affinity (Kd=7.2±0.7 pM) and mean density of 15±1 fmol/mg protein (n=5). The time dependent binding of ^{125}I-(-)-CYP at a free concentration of 7 pM indicated that it took 5 hours of incubation to reach equilibrium. Equilibration time could be reduced to 2 hours by increasing the ligand concentration to 18 pM.
The binding of ^{125}I-(-)-CYP (16 pM) to β-adrenoceptors in sections of rat nasal mucosa was stereoselectively inhibited by stereoisomers of propranolol, (-)-propranolol having 460 times greater affinity than

(+)-propranolol in displacing ^{125}I-(-)-CYP from its binding sites (table 4.1).

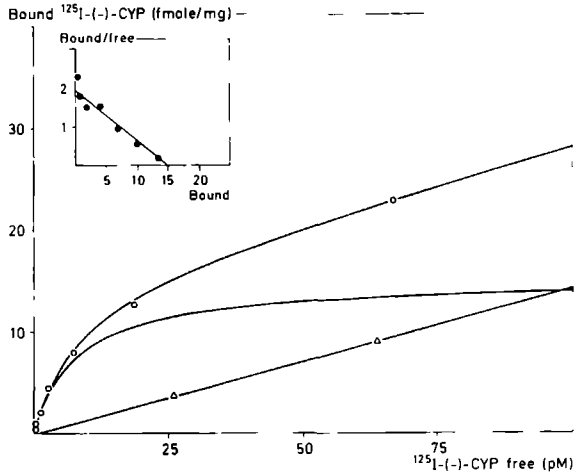

Fig. 4.4: Specific ^{125}I-(-)-CYP binding to cryostat sections of rat nasal mucosa. Total binding (0) and non-specific binding (Δ) were determined in the absence and presence of 2 μM (±)-propranolol. Inset: Scatchard plot of the specific part of the binding. Data shown are the means of triplicates from a representative experiment.

Inhibition of ^{125}I-(-)-CYP by $LK_{203-030}$ and $ICI_{118,551}$ demonstrated the presence of a heterogeneous β-adrenoceptor population, $LK_{203-030}$ detected 43±11% and $ICI_{118,551}$ 45±5% β_1-adrenoceptors respectively (fig. 4.5).
In all binding and competition experiments incubations were terminated by rinses in buffer. It appears that rinses of 4 x 15 min reduce the non-specific binding of the radioligand to the sections and result in an optimal ratio specific to non-specific binding.

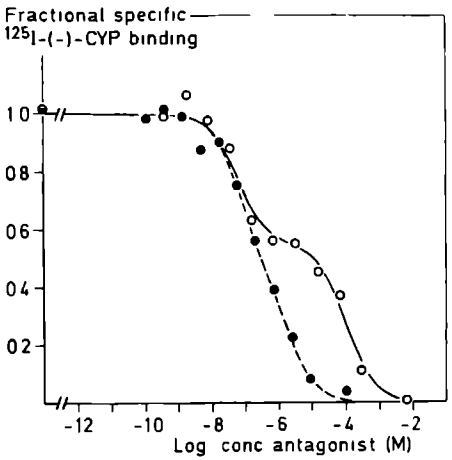

Fig. 4.5:
Inhibition of specific ^{125}I-(-)-CYP binding by the antagonists $LK_{203-030}$ (O) and $ICI_{118,551}$ (●). Cryostat sections of rat nasal mucosa were incubated at 37^{0}C for 120 min with 20 pM ^{125}I-(-)-CYP in the presence or absence of competing agents. Data shown are the means of triplicates from a representative experiment.

Autoradiography.

After 2 months exposure the autoradiographs showed a dense labelling of the nasal epithelium and the glandular excretory ducts (fig. 4.6 A and 4.7 A) with ^{125}I-(-)-CYP. Autoradiographs made from sections incubated in the presence of 2 μM (±)-propranolol in addition to the radioligand showed only a faint homogeneous labelling of the nasal mucosa (fig. 4.6 B and 4.7 B). The glandular acini showed a very slight labelling, which hardly could be distinguished from the non-specific labelling, whereas the connective tissue and the blood vessels were devoid of silver grains. In order to localize β-adrenoceptor subtypes, series of consecutive sections were incubated with 20 pM ^{125}I-(-)-CYP, in the presence of 2 μM (±)-propranolol; 0.1, 0.5 or 1.0 μM $ICI_{118,551}$, or in the presence of 5, 30 or 55 μM $LK_{203-030}$. The autoradiograms generated with high concentrations $ICI_{118,551}$ (1.0 μM) and $LK_{203-030}$ (30 and 55 μM) were indentical to those obtained with 2 μM (±)-propranolol. The autoradiograms generated with a low concentration $ICI_{118,551}$ (0.1 μM) were similar to those obtained with ^{125}I-(-)-CYP alone. In autoradiograms obtained with 0.5 μM $ICI_{118,551}$ or 5 μM $LK_{203,030}$ β_1- or β_2-adrenoceptors were labelled preferentially. Those autoradiograms showed a reduction of specific labelling in both epithelium and striated

excretory ducts indicating that both β_1- and β_2-adrenoceptors are present in these structures.

4.5. Discussion

Radioligand receptor binding studies with the antagonist ^{125}I-(-)-CYP have been used to demonstrate β-adrenoceptors in homogenates of many tissues (Stiles et al., 1984). In this study binding studies were performed to homogenates but also to cryostat sections of rat nasal mucosa. This in vitro labelling of the sections combined with autoradiography give the opportunity to localize the β-adrenoceptors.
Until now, in vitro autoradiography has been mainly applied to the localization of high receptor densities in the brain (Palacios and Kuhar, 1982) or in the lower airways (Carstairs et al., 1985). Recently, an in vitro method has been developed to localize low muscarinic receptor densities in the rat nasal glands (van Megen et al., 1988a). This method was applied to localize β-adrenoceptors in rat nasal mucosa. Specific ^{125}I-(-)-CYP binding to β-adrenoceptors in homogenates or in cryostat sections of rat nasal mucosa was saturable and of high affinity. The Kd-values of the binding to homogenates (5.0±0.4 pM) and cryostat sections (7.2±0.7 pM) were similar to those determined in other tissues (Stiles et al., 1984; Carstairs et al., 1985; van Koppen et al., 1987). No differences between Kd-values for ^{125}I-(-)-CYP binding to homogenates or cryostat sections have also been observed in rat kidney (Summers et al., 1985) and human lung (Carstairs et al., 1985). The β-adrenoceptor density per mg protein is higher in homogenates (204±12 fmol/mg protein) than in sections (15.2±0.4 fmol/mg protein). This discrepancy may be due to differences in protein contents; homogenates consist of a relatively pure membrane fraction, whereas sections contain all tissue contituents. The presence of β-adrenoceptors in homogenates of nasal mucosa of guinea pig (Ishibe et al., 1985; Konno et al., 1987) and man (Ishibe et al., 1983; Konno et al., 1987) has been demonstrated earlier using the antagonist ^{3}H-Dihydroalprenolol. The present study comprises also pharmacological characteristics of the ^{125}I-(-)-CYP binding and the distribution of β-adrenoceptor subtypes in rat nasal mucosa.

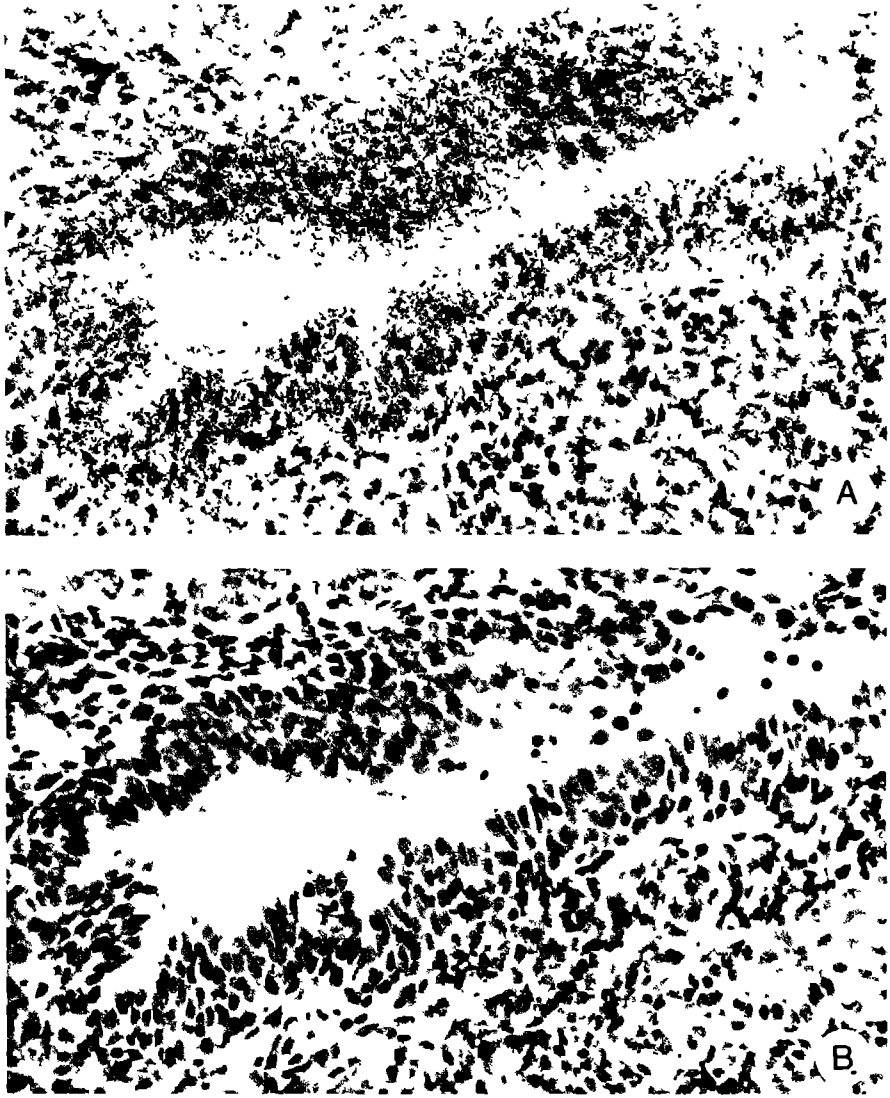

Fig. 4.6:
Autoradiograms of 0.5% glutardialdehyde fixed cryostat sections (10 μm) of rat nasal mucosa after incubation with 20 pM ^{125}I-(-)-CYP (A) or with 2 μM (±)-propranolol in addition to the radioligand (B). The autoradiograms show specific labelling of the nasal epithelium. Staining with methylgreen pyronin (1%) x 800.

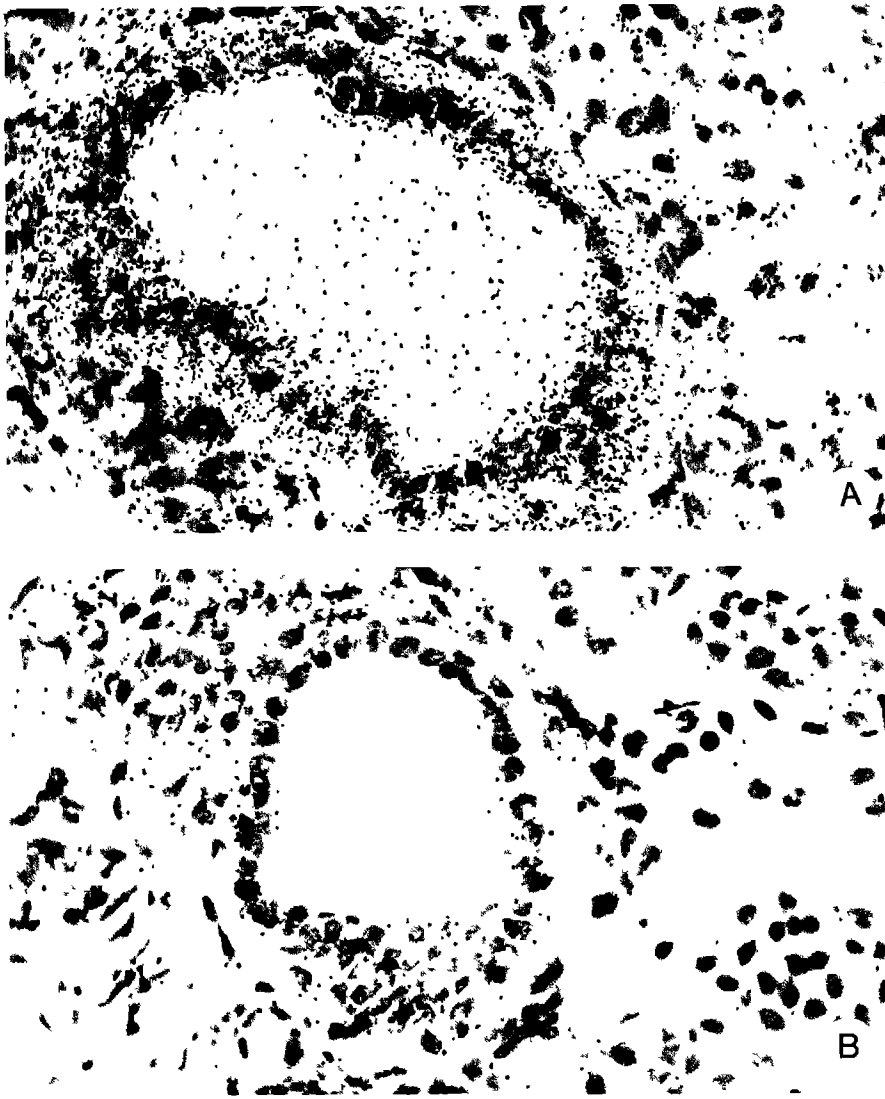

Fig. 4.7:
Autoradiograms of 0.5% glutardialdehyde fixed cryostat sections (10 μm) of rat nasal mucosa after incubation with 20 pM ^{125}I-(-)-CYP (A) or with 2 μM (±)-propranolol in addition to the radioligand (B). The autoradiograms show specific labelling of the nasal glandular excretory ducts. Staining with methylgreen pyronin (1%).x800.

The time dependent ^{125}I-(-)-CYP (3 pM) binding to homogenates indicated that equilibrium was reached after 120 min incubation, which is in agreement with the $t_{½}$ (about 35 min). In cryostat sections equilibrium was only reached after 5 hours using a low concentration of ^{125}I-(-)-CYP (7 pM). As expected by increasing the ^{125}I-(-)-CYP concentration to 18 pM, the equilibrium time was reduced to 2 hours. The lower association rate of ^{125}I-(-)-CYP in cryostat sections in comparison with homogenates may be due to a slow diffusion of the radioligand in and out the sections. Unexpectedly, the Scatchard plot of the specific binding was linear after 2 hours incubation and the Kd value was in agreement with that determined in homogenates. For the in vitro autoradiographic experiments a concentration of 20 pM ^{125}I-(-)-CYP was used. This concentration ensured a rapid equilibration, 80% saturation of the binding sites and a favourable specific to non-specific ratio.

The presence of β-adrenoceptors has been connected with adrenergic innervation. According to Ariëns and Simonis (1983) β_1-adrenoceptors are meant for the neurotransmitter noradrenaline and β_2-adrenoceptors for the hormone adrenaline. Inhibition experiments with $LK_{203-030}$, reported to have a 500-1000 fold β_1-selectively (Milavec-Krizman et al., 1985), demonstrated a heterogeneous population of β-adrenoceptors consisting of 50% and 43% β_1-adrenoceptors in homogenates and cryostat sections of rat nasal mucosa, respectively. The Ki-values correspond well with those reported in homogenates of rat kidney (Taylor et al., 1984; Milavec-Krizman et al., 1985). There was a slight discrepancy in the distribution of β-adrenoceptors measured by the β_2-selective antagonist $ICI_{118,551}$. The β_1-adrenoceptor population in cryostat sections (45%) was in agreement with that determined with $LK_{203-030}$ (43%) but the β_1-adrenoceptor population in homogenates (63%) was somewhat higher than that determined with $LK_{203-030}$ (50%). This apparent discrepancy may be due to the low selectivity (65-fold) of $ICI_{118,551}$ (Milavec-Krizman et al., 1985). The Ki-values of the inhibition of ^{125}I-(-)-CYP binding with $ICI_{118,551}$ were in agreement with those reported in homogenates of rat kidney (Milavec-Krizman et al., 1985) and in sections of human lung (Carstairs et al., 1985).

The present results show that in homogenates of rat nasal mucosa the β-adrenergic agonist isoprenaline binds to two different states, a high (30±3%) and low affinity agonist state. The Ki-values were in agreement with those described in other tissues (Kent et al., 1980; Brodde, 1982; Stiles et al., 1984). It has been accepted that the high affinity complex consists of agonist, receptor and guaninenucleotide binding protein (Harden et al., 1982; Stiles et al., 1984; Levitzki, 1986). Guaninenucleotides appeared to convert the high affinity states into low affinity states of the receptor (Brodde, 1982; Stiles et al., 1984; Severne et al., 1987).

In the presence of 5.10^{-4} M Gpp(NH)p a steepening of the inhibition curve, but no complete conversion of high into low affinity states was observed. Since the protease inhibitor PMSF had no effect on the inhibition curves, proteolytic activity is not responsible for the incomplete conversion. Furthermore, the two inhibition curves in the absence and presence of Gpp(NH)p were analysed simultaneously as a pair according to two models:

a) a model with the restriction that the affinity constants for the high and low affinity state were equal in the absence or presence of Gpp(NH)p, while the fraction receptors in the high affinity state was optimized independently for both situations.

b) a model with no restrictions i.e. the affinity constants as well as the fraction receptors in the high affinity state were optimized independently in the absence or presence of Gpp(NH)p.

In the second analysis the residual sum of squares was significantly lower (F-test; $p<0.05$) indicating that no simple conversion of receptors from the high to the low affinity state occurred but rather a transfer from one set of high and low affinity states to another set of high and low affinity states accompanied by an overall lower affinity. Complete conversion has neither been demonstrated in rat liver (Dax et al., 1986) in trachea smooth muscle (van Koppen et al., 1987) and in rat heart (Christ et al., 1988).

After an exposure time of two months the autoradiographs of 10 μm sections of the rat nasal mucosa showed specific ^{125}I-(-)-CYP labelling of the epithelium. This finding was in agreement with a histochemically

weak positive noradrenalin reaction (Klaassen et al., 1988). Beta-adrenoceptors have also been demonstrated in the epithelium of the trachea (Davis et al., 1979; Agrawal et al., 1987) and lung (Carstairs et al., 1985). It has been suggested that these receptors in airway epithelium play a role in the mucociliary transport, active iontransport or the production of epithelium derived relaxatory factors (Davis et al., 1979; Carstairs et al., 1985; Agrawal et al., 1987).

The autoradiographs showed also a specific ^{125}I-(-)-CYP labelling of the striated excretory ducts of the nasal glands as described before (van Megen et al., 1988b). The presence of β-adrenoceptors in striated excretory ducts has also been demonstrated in the salivary glands, where they control the ion and water content (Schneyer et al., 1972). It has been suggested that the striated excretory ducts of the nasal glands have a similar function (Phipps, 1981).

The autoradiographs demonstrated a slight labelling of the nasal glandular acini. No evidence for adrenergic innervation of the acini could be observed in histochemical experiments (Grote et al., 1975; Vecerina et al., 1983). A small population of β-adrenoceptors has been demonstrated in submucosal glands of the human lung (Carstairs et al., 1985).

From these and previous experiments (van Megen et al., 1988a; van Megen et al., 1988b) it may be concluded that the production of glycoproteins by the acini is mainly under parasympathetic control, whereas the regulation of the ion and water content of the nasal fluid by the excretory ducts is under both sympathetic and parasympathetic control.

In this study, the autoradiographs did not show specific labelling of β-adrenoceptors in nasal blood vessels. This finding is in agreement with several physiological studies (Hall and Jackson, 1968; Anggård and Densert, 1974; Svensson et al., 1980). However, other physiological studies demonstrated β-adrenoceptors in resistance (Malm, 1974) and capacitance vessels (Malm, 1974; Hiley et al., 1978).

Clinical pharmacological studies refer to the anti-allergic effects of β-adrenergic agonists by inhibiting histamine release from mast cells (Kaliner and Austen, 1975). Botana (1987) demonstrated β-adrenoceptors on pleural and peritoneal mast cells. However, only a few mast cells were

present in rat nasal mucosa. No specific ^{125}I-(-)-CYP labelling in these cells could be observed.

The radioligand receptor binding studies and in vitro autoradiography suggested the presence of a mixture of β_1- and β_2-adrenoceptor populations in the nasal epithelium and in the glandular excretory ducts. The coexistence of β-adrenoceptors subtypes has also been observed in rat kidney (Summers et al., 1985). In human lung epithelium, a homogeneous β_2-adrenoceptor population was observed. However, the authors suggested that the epithelium was probably contaminated with smooth muscle consisting of pure β_2-adrenoceptors (Carstairs et al., 1985).

In summary, β-adrenoceptors have been demonstrated and characterized in homogenates as well as in cryostat sections of rat nasal mucosa. Furthermore, the in vitro labelling on cryostat sections was combined with autoradiography. The radioligand receptor binding experiments and the in vitro autoradiography demonstrated both β_1- and β_2-adrenoceptors in the epithelium and glandular excretory ducts. In the future, both techniques will be applied to human nasal mucosa for studying the supposed changes in the distribution pattern of receptors under pathological conditions such as nasal hyperreactivity.

Acknowledgement

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DEMONSTRATION OF ALPHA$_1$-ADRENOCEPTORS IN RAT NASAL MUCOSA.

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5.1. Abstract

^{3}H-Prazosin was used to demonstrate α_1-adrenoceptors in rat nasal mucosa. Specific binding is saturable and occurs to a homogeneous class of binding sites with high affinity (Kd=0.07±0.01 nM) and with a receptor density of 0.36±0.02 pmol/g tissue or 14±1 fmol/mg protein. Kinetic experiments resulted in a Kd-value of 0.03 nM. The binding is stereoselectively inhibited by epinephrine enantiomers. The antagonist prazosin inhibits ^{3}H-Prazosin binding with high affinity, in contrast to yohimbine, classifying the binding sites as α_1-adrenoceptors. Inhibition experiments with WB4101 indicated the presence of α_{1a}- (31±9%) and α_{1b}-adrenoceptor subtypes in the rat nasal mucosa. The order of potencies of agonists determined in competition experiments was (-)epinephrine > (+)epinephrine > (-)phenylephrine.

5.2. Introduction

Physiological experiments have demonstrated that adrenergic receptors are involved in the vasomotor activities of the nasal mucosa (Eccles and Wilson, 1974; Grote et al., 1975; Jackson, 1979; Jackson, 1980; Ichimura and Jackson, 1984). Although nasal secretion has been proven to be mainly under parasympathetic control, it has been suggested that

α-adrenoceptors play a role in the regulation of nasal secretory activity (Malm et al., 1983).

Neurohistochemical studies also point to the presence of a dense adrenergic innervation of nasal blood vessels, especially the sinusoids, regulating the swelling and shrinkage of the nasal mucosa (Grote et al., 1975; Anggård and Densert, 1974; Vecerina et al., 1983). Radioligand receptor binding studies give the opportunity of investigating the biochemical characteristics, densities and subclasses of adrenoceptors. The α-adrenoceptors have been divided into α_1- and α_2-adrenoceptors based on their pharmacological profiles (Berthelsen and Pettinger, 1977; Hoffman et al., 1979). In this study we have demonstrated the presence of α_1-adrenoceptors in the rat nasal mucosa with the radioligand binding technique using the selective antagonist ^{3}H-Prazosin (Timmermans and van Zwieten, 1981; Johansson, 1984). Recently, the presence of α_1-adrenoceptor subtypes have been reported (Morrow and Creese, 1986; Han et al., 1987; Minneman et al., 1988). It appeared that the antagonist WB4101 distinguishes two α_1-adrenoceptor binding sites, one high (α_{1a}) and one low (α_{1b}) affinity binding site. We used this antagonist to demonstrate the presence of α_1-adrenoceptor subclasses in rat nasal mucosa.

5.3. Materials and methods

Chemicals

^{3}H-Prazosin was purchased from New England Nuclear (spec. activity: 18.8 Ci/mmol), Doorn, The Netherlands. (+) and (-)epinephrine bitartrate were gifts from Sterling Winthrop, Haarlem, The Netherlands. Prazosin was a gift from Pfizer, New York and phentolamine from Ciba-Geigy, N.J. Yohimbine-HCl and (-)phenylephrine were obtained from Sigma Chemical Co., St. Louis, MO, USA and WB4101 from Amersham International, England. All other chemicals were of analytical grade.

Membrane Preparations

Nasal mucosae of Wistar rats (body weight about 250 g) were carefully dissected, immediately frozen (acetone/CO_2 bath) and stored at -80°C. The

tissue was homogenized in ice-cold Tris/HCl buffer (50 mM Tris; 120 mM NaCl; 5 mM KCl; 2 mM $CaCl_2$; 1 mM $MgCl_2$, pH 7.4) with an Ultraturrax for 2x10 seconds. The homogenate was centrifuged at 1000xg for 5 min, the supernatant was collected and centrifuged at 50,000xg for 15 min (4°C). The resulting pellet was washed 3 times in ice-cold Tris/HCl buffer by resuspension and centrifugation (50,000xg, 15 min). The final pellet was resuspended by Potter homogenization in buffer. Protein determination was performed according to Lowry using bovine serum albumin as a standard.

Radioligand Receptor Binding Assay

Receptor binding studies were carried out in glass tubes containing 40 µl ^{3}H-Prazosin, 40 µl Tris/HCl buffer and 320 µl tissue homogenate corresponding to a final concentration of 25 mg/ml tissue. Instead of the buffer, phentolamine was added to a final concentration of 12 µM for the determination of the non-specific binding. The homogenates were incubated at 25°C for 40 min. Incubations were terminated by rapid dilution with 2x2 ml ice-cold buffer and filtration through Whatman GF/C filters under vacuum, followed by 3x5 ml rinses of the filter with buffer. Radio-activity collected on each filter was determined by liquid scintillation counting after the addition of 10 ml Aqua luma (Lumac). The binding parameters were calculated by subjecting the data to a non-linear least square curve fitting procedure using the Gauss-Newton algorithm (Fletcher and Powell, 1963) comparable with the LIGAND program (Munson and Rodbard, 1980). Inhibition curves were analysed according to a one or two binding sites model.

5.4. Results

Specific binding of ^{3}H-Prazosin to rat nasal mucosa membranes was saturable and of high affinity (fig. 5.1). A Scatchard representation (insert) points to a homogeneous population of binding sites. Analysis of the data from 6 experiments results in a mean equilibrium dissociation constant (Kd) of 0.07±0.01 nM and a total receptor density (Bmax) of 0.36±0.02 pmol/g tissue. Tissue protein content of the homogenates has been determined in 4 out of 6 experiments, resulting in 25±4 mg

protein/g tissue. With this converting factor the receptor density has been expressed in fmol/mg protein (Bmax=14±1 fmol/mg protein).

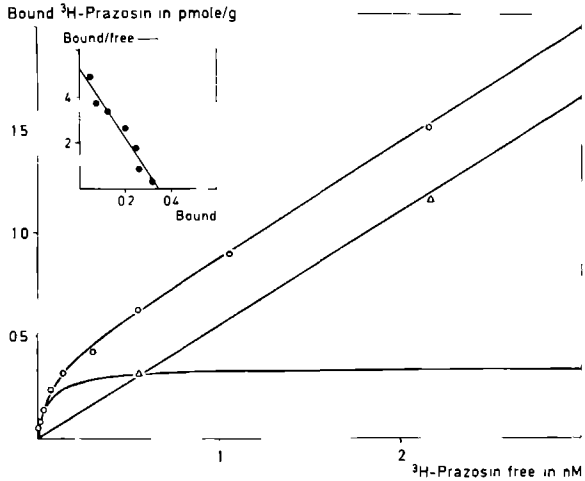

Fig. 5.1: Specific binding of ^{3}H-Prazosin to rat nasal mucosa membranes (25 mg/ml). Total binding (O) and non-specific binding (Δ), determined in the presence of 12 μM phentolamine. The line without points represents the specific ^{3}H-Prazosin binding. Inset: Scatchard plot of the specific part of the binding.

^{3}H-Prazosin binding was linear with tissue concentrations in the range of 15-45 mg/ml. If a tissue concentration below 10 mg/ml was used specific binding could hardly be demonstrated. Using a tissue concentration of 25 mg/ml reproducible specific binding was obtained. Furthermore, non-specific binding could be reduced to a level of 50% at 1 nM ^{3}H-Prazosin applying filtration instead of centrifugation after the incubation. Extending the wash-procedure of the membrane pellets (4 washes instead of one wash) resulted in a 10 % reduction of the non-specific binding.

As illustrated in figure 5.2, the specific binding of ^{3}H-Prazosin to rat nasal mucosa was rapid, 95% equilibrium being reached in approximately 40 min. Specific binding was reversible upon addition of 12 μM phentolamine.

The association (k_1) and dissociation (k_{-1}) rate constants were 0.50±0.13 10^9 $M^{-1}min^{-1}$ and 0.016±0.002 min^{-1} (n=3). The corresponding kinetic Kd (k_{-1}/k_1) value of 0.03 nM was somewhat lower than the equilibrium Kd-value.

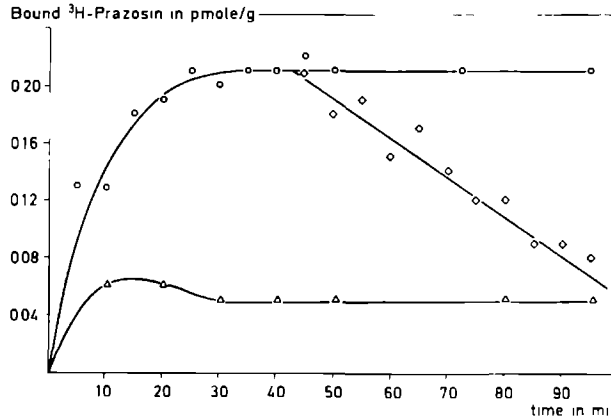

Fig. 5.2: Time course of association and dissociation of ^{3}H-Prazosin binding to rat nasal mucosa. Incubations were performed with 0.1 nM ^{3}H-Prazosin at 25°C. Dissociation was induced by addition of 12 µM phentolamine after 40 min incubation. Total binding (O), non-specific binding (Δ), dissociation (◇).

Adrenergic antagonists competed for the ^{3}H-Prazosin binding sites in rat nasal mucosa with an order of potencies clearly indicative of α_1-specificity (fig. 5.3). Prazosin (Ki=0.11 and 0.19 nM) was 1000 times more potent than yohimbine (Ki=0.11 and 0.14 µM). Inhibition experiments with the antagonist WB4101 showed shallow inhibition curves (fig. 5.3). The curves could best be fitted with a two binding sites model (Ki_1=0.18±0.15 nM; Ki_2=5.3±0.6 nM) and indicated the presence of α_{1a}-(31±9%) and α_{1b}-adrenoceptors. The adrenergic agonists competed for the ^{3}H-Prazosin binding sites in the following order of potency: (-)epinephrine (Ki=1.9 and 2.8 nM), (+)epinephrine (Ki=52 and 71 nM), (-)phenylephrine (Ki=2.8 and 5.4 µM)(fig. 5.4). All agonist inhibition

curves displayed a normal slope (one binding site). The ^{3}H-Prazosin binding is stereoselectively inhibited by the enantiomers of epinephrine, (-)epinephrine having 23 times greater affinity than (+)epinephrine.

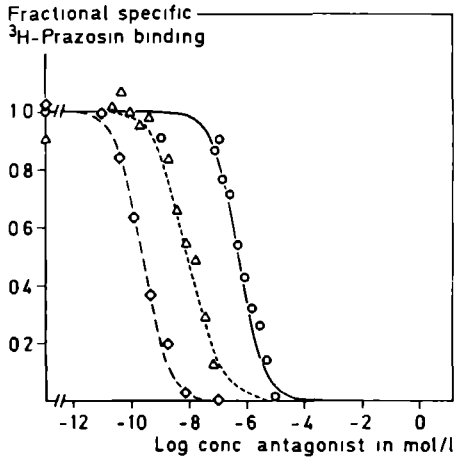

Fig. 5.3:
Inhibition of specific ^{3}H-Prazosin binding by the antagonists prazosin (◇), yohimbine (○) and WB4101 (△). Nasal mucosa membranes were incubated at 25°C for 40 min with 0.2 nM ^{3}H-Prazosin in the absence or presence of competing agents. Data shown are the means of triplicates from a representative experiment.

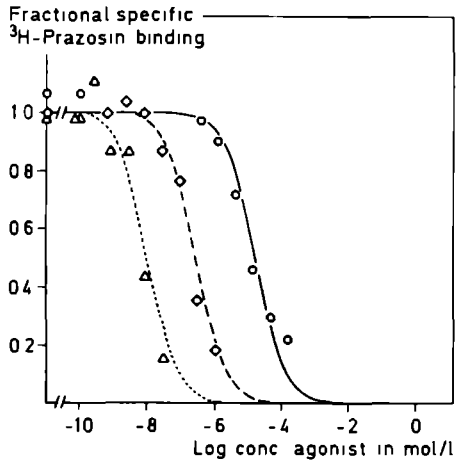

Fig. 5.4:
Inhibition of specific ^{3}H-Prazosin binding by the agonists (-)epinephrine (△), (+)epinephrine (◇) and (-)phenylephrine (○). Nasal mucosa membranes were incubated at 25°C for 40 min with 0.2 nM ^{3}H-Prazosin in the absence or presence of competing agents. Data shown are the means of triplicates from a representative experiment.

5.5. Discussion

Appropriate conditions, especially a tissue concentration above 10 mg/ml, were essential to observe specific ^{3}H-Prazosin binding to α_1-adrenoceptors in rat nasal mucosa. This may be due to the unfavourable ratio specific to non-specific binding. Notably, the α_1-adrenoceptor density (14 fmol/mg protein) was low in contrast with the density of 200 fmol/mg protein in rat cerebral cortex (Diop et al., 1987) or 177 fmol/mg protein in rat mesenteric arteries (Agrawal and Daniel, 1985).

Specific binding of ^{3}H-Prazosin to rat nasal mucosa was of high affinity with a Kd-value of 0.07±0.01 nM (n=6). The binding occurs to a homogeneous class of binding sites with a receptor density of 0.36±0.02 pmol/g tissue or 14±1 fmol/mg protein. The Kd-values in the present study are in agreement with those found in other tissues of the rat (Guicheney and Meyer, 1981; Agrawal and Daniel, 1985; Rattigan et al., 1986; Diop et al., 1987). ^{3}H-Prazosin binding was demonstrated in nasal mucosa of guinea pig (Kd=0.37 nM (Ishibe et al., 1985) and Kd=2.63 nM (Konno et al., 1986)) and man (Kd=0.31 nM (Ishibe et al., 1983)). The Kd-values reported were higher than those found in this study. To rule out a possible species difference we determined the dissociation constant of ^{3}H-Prazosin binding in guinea pig nasal mucosa, which appeared to be similar. As we applied the receptor assay method of Ishibe et al. (1983; 1985) only small differences in methodology, like the use of glass instead of plastic incubation tubes, can account for the discrepancy in dissociation constant.

The saturability, stereoselectivity, reversibility and kinetics of the ^{3}H-Prazosin binding indicated that ^{3}H-Prazosin binds to α_1-adrenoceptors in rat nasal mucosa. Competition experiments confirmed the α_1-adrenergic characteristics of the binding sites since ^{3}H-Prazosin was 1000 times more potent than yohimbine (Timmermans and van Zwieten, 1981; Johansson, 1984). Competition experiments with WB4101 indicated the presence of α_{1a}- (31±9%) and α_{1b}-adrenoceptors. The Ki-values for WB4101 at the high (α_{1a}) and the low (α_{1b}) affinity binding sites were in agreement with

those in heart, cortex and kidney (Morrow and Creese, 1986; Minneman et al., 1988). It has been suggested that α_{1a}-adrenoceptors mediate opening of the Ca^{2+}-channels, whereas the α_{1b}-adrenoceptors stimulate the inositol phospholipid metabolism (Morrow and Creese, 1986; Han et al., 1987; Minneman et al., 1988). The relative potency of agonists was in agreement with those of other studies (Barnes et al., 1979; Snavely and Insel, 1982). The competition curves of the ^{3}H-Prazosin binding followed a sigmoid shape. This is in agreement with the homogeneity of agonist binding sites observed in other tissues (Barnes et al., 1979; Guicheney and Meyer, 1981; Agrawal and Daniel, 1985; Rattigan et al., 1986). However, several studies have demonstrated biphasic agonist competition curves, indicating heterogeneous agonist binding sites (Adams et al., 1986; Hieble et al., 1986; Jagadeesh and Deth, 1987).

Physiological and histochemical experiments have provided evidence for the presence of α_1-adrenoceptors in smooth muscles of nasal blood vessels which mediate vasoconstriction (Anggård and Densert, 1974; Grote et al., 1975; Vecerina et al., 1983; Ichimura and Jackson, 1984). In studies of the lower airways it has also been suggested that these receptors are present on mast cells, where they regulate mediator release (Konno et al., 1986). Autoradiographic experiments will be needed to localize these ^{3}H-Prazosin binding sites.

This study may contribute to a better understanding of the mechanisms of clinically used drugs. Commonly used nasal decongestants stimulate both α_1- and α_2-adrenoceptors. However, Andersson and Bende (1984) demonstrated that α_1-acting drugs, in contrast to α_2-acting drugs, did not reduce the nasal blood flow. Therefore, a nasal decongestant with only an α_1-adrenoceptor stimulating effect might be preferable to a drug which also acts on α_2-adrenoceptors, provided that the decongestive effects are similar. It has been reported that the nasal decongestant Abbott-57219 mediates selective α_1-vasoconstriction but not constriction of the resistance vessels (Kyncl et al., 1987).

In the present study a radioreceptor assay has been adapted for the demonstration and characterization of α_1-adrenoceptors in rat nasal mucosa. This assay will be applied to human nasal mucosa in order to study the supposed changes in the distribution pattern of receptors under

pathological conditions such as nasal hyperreactivity.

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ALPHA$_2$-ADRENOCEPTORS IN HOMOGENATES OF RAT NASAL MUCOSA.

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6.1. Abstract

The specific binding of ^{3}H-Rauwolscine to membranes of rat nasal mucosa was saturable, stereoselective and of high affinity. In 7 out of 14 experiments the Scatchard plot points to an homogeneous population of binding sites (Kd=3.6±0.6 nM; Bmax=5.1±0.7 pmol/g). In the other experiments ^{3}H-Rauwolscine binding was significantly better fitted according to a two binding sites model with high (Kd=0.20±0.08 nM; Bmax=0.5±0.1 pmol/g) and low affinity binding sites (Kd=17±6 nM; Bmax=11±5 pmol/g). Investigation of the non-specific and filter binding suggested that the appearance of two binding sites may be an artefact of an inappropriate definition of the specific binding due to non-linear, non-specific binding.
Inhibition of ^{3}H-Rauwolscine binding with the subtype selective antagonist prazosin suggested the presence of α_2-adrenoceptor subclasses in rat nasal mucosa. All full agonist inhibition curves demonstrated high and low affinity agonist binding sites. In the presence of guanine nucleotides a monophasic (-)-epinephrine inhibition curve was obtained.

6.2. Introduction

Physiological and morphological experiments have shown that the nasal vascular structures are innervated by sympathetic (adrenergic) nerves (Anggård and Densert, 1974; Eccles and Wilson, 1974; Grote et al., 1975; Ichimura and Jackson, 1984; Vecerina et al., 1983; Berridge and Roach,

1986). Although nasal secretion has been proven to be mainly under parasympathetic control, it has been suggested that adrenoceptors play a role in the regulation of the nasal secretory activity (Malm et al., 1983).

Clinically used nasal decongestants points to the presence of α-adrenoceptors in human nasal mucosa (Andersson and Bende, 1984). Radioligand receptor binding studies can be used to investigate the biochemical characteristics, densities and subclasses of adrenoceptors. The α-adrenoceptors have been divided into α_1- and α_2-adrenoceptors based on their pharmacological profiles (Berthelsen and Pettinger, 1977; Hoffman et al., 1979). The α_1-adrenoceptors have already been demonstrated and characterized in homogenates of rat (van Megen et al., 1989) and guinea pig nasal mucosa (Ishibe et al., 1985). No quantitative data of α_2-adrenoceptors in rat nasal mucosa are available. In this study we have demonstrated the presence of α_2-adrenoceptors using the selective antagonist ^{3}H-Rauwolscine as radioligand (Timmermans and van Zwieten, 1982; Johansson, 1984). Alpha$_2$-adrenoceptor subtypes have been proposed on the basis of the affinity for prazosin; α_{2A}- and α_{2B}-adrenoceptors having low and high affinity respectively (Bylund, 1985; Nahorski et al., 1985). In this study, the characteristics of the α_2-adrenoceptors in regard to affinity, receptor density, stereoselectivity, classification into their subtypes and the inhibitory pattern of the antagonist binding by agonists have been demonstrated.

6.3. Materials and methods

^{3}H-Rauwolscine was purchased from New England Nuclear (spec. activity 73.5 Ci/mmol), Doorn, The Netherlands. (+)- and (-)-epinephrine bitartrate were gifts from Sterling Winthrop, Haarlem, The Netherlands. Prazosin was a gift from Pfizer, New York and phentolamine from Ciba-Geigy, N.J. Yohimbine-HCl, clonidine, oxymetazoline and Gpp(NH)p (5'-guanylylimidodiphosphate) were obtained from Sigma Chemical Co., St. Louis, M.O., U.S.A. All other chemicals were of analytical grade.

Membrane preparations

The nasal mucosae from Wistar rats (body weight about 250 g) were carefully dissected, immediately frozen (acetone/CO_2 bath) and stored at -80°C. The tissue was homogenized in ice-cold Tris/EDTA buffer (50 mM Tris, 1 mM EDTA) with an ultraturrax for 2x10 sec. The homogenate was centrifuged at 1000xg for 5 min, the supernatant was collected and centrifuged at 100,000xg for 60 min (4°C). The resulting pellet was resuspended by Potter homogenization in buffer. Protein determination was performed according to Lowry using bovine serum albumin as a standard.

Receptor binding studies.

Receptor binding studies were carried out in glass tubes, containing 40 µl ^{3}H-Rauwolscine, 40 µl Tris/EDTA buffer and 320 µl tissue homogenate corresponding to a final concentration of 10 mg/ml. Instead of the buffer, phentolamine was added to a final concentration of 12 µM in buffer for the determination of the non-specific binding. The homogenates were incubated at 25°C for 20 min. Incubations were terminated by rapid dilution with 2x2 ml ice-cold buffer and filtration through Whatman GF/C filters under vacuum followed by 3x5 ml rinses of the filter with buffer. Radioactivity on each filter was determined by liquid scintillation counting after the addition of 10 ml Aqua Luma (Lumac). The binding parameters were calculated by subjecting the data to a non-linear least squares curve fitting procedure using the Gauss Newton algorithm (Fletcher and Powell, 1963). Antagonist binding and inhibition curves were analysed according to a one or two binding sites model.

6.4. Results

Specific binding of ^{3}H-Rauwolscine to membranes of rat nasal mucosa was saturable and of high affinity (fig. 6.1). Analysis of the data demonstrated a mean dissociation constant (Kd) of 4.1±0.5 nM and a receptor density (Bmax) of 5.9±0.5 pmol/g tissue corresponding with 236±32 fmol/mg protein (n=14).

The ^{3}H-Rauwolscine (0.5 nM) binding to rat nasal mucosa was linearly dependent on tissue concentration between 5-45 mg/ml.

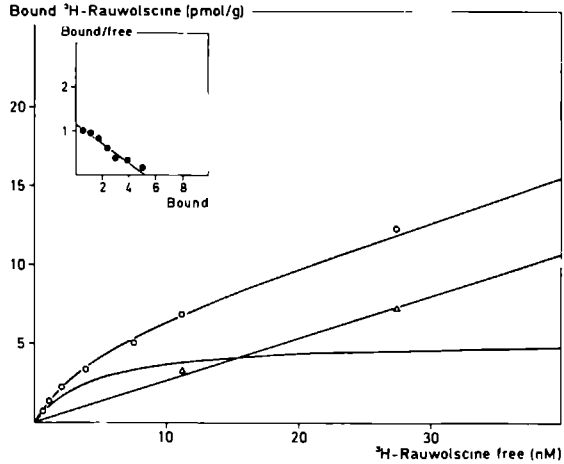

Fig. 6.1: Specific binding of ^{3}H-Rauwolscine to rat nasal mucosa membranes (25 mg/ml). Total binding (O) and non-specific binding (△), determined in the presence of 12 μM phentolamine. The line without points represents the specific ^{3}H-Rauwolscine binding. Inset: Scatchard plot of the specific binding.

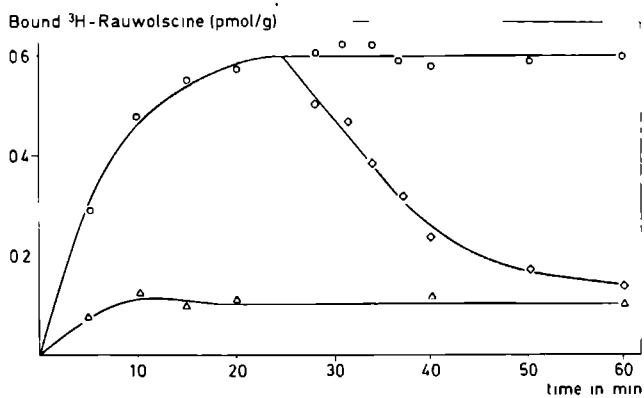

Fig. 6.2: Time course of association and dissociation of ^{3}H-Rauwolscine binding to rat nasal mucosa membranes. Incubations were performed with 0.5 nM ^{3}H-Rauwolscine at 25°C. Dissociation was induced by addition of 12 μM phentolamine after 25 min incubation. Total binding (O) non-specific binding (△) dissociation (◇).

The time dependent association and dissociation from α_2-adrenoceptors in rat nasal mucosa is illustrated in fig. 6.2. The specific binding of ^{3}H-Rauwolscine (0.5 nM) was rapid, 95% equilibrium being reached in approximately 20 min. Specific binding was reversible upon addition of 12 μM phentolamine. If ln(Bt/Beq) was plotted versus time, the dissociation reaction followed first order kinetics, indicating the presence of an homogeneous class of binding sites. The association (k_1) and dissociation (k_{-1}) constants were 0.023±0.003 $nM^{-1}min^{-1}$ and 0.032±0.008 min^{-1} respectively; the calculated Kd-value ($Kd=k_{-1}/k_1$) of 1.4±0.3 nM (n=3) was somewhat lower than the equilibrium Kd-value.

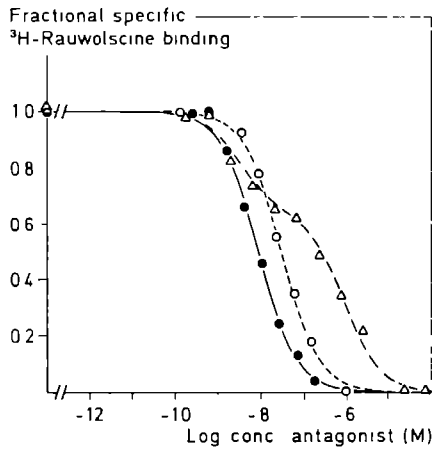

Fig. 6.3:
Inhibition of the specific ^{3}H-Rauwolscine binding by the antagonists phentolamine (●), yohimbine (○) and prazosin (△). Nasal mucosa membranes were incubated at 25°C for 20 min with 8 nM ^{3}H-Rauwolscine in the absence or presence of competing agents. The curve of prazosin could significantly better fitted according to a two binding sites model whereas the curves of phentolamine and yohimbine could adequately be fitted according to a one binding site model. Data shown are the means of triplicates from a representative experiment.

The subtype selective antagonist prazosin was used to define the proportion of α_2-adrenoceptor subtypes. The inhibition curve was shallow and could be significantly better fitted according to a two binding site model, suggesting the presence of α_2-adrenoceptor subclasses (table 6.1; fig. 6.3). Adrenergic antagonists competed for the ^{3}H-Rauwolscine binding sites in rat nasal mucosa with the following order of potency; phentolamine > yohimbine > prazosin (low affinity) (table 6.1; fig. 6.3). Inhibiton curves of the partial agonist clonidine displayed a monophasic character, whereas the full agonist oxymetazoline displayed a biphasic

character, demonstrating 52±5% high affinity states (table 6.1; fig. 6.4). Inhibition curves of the full agonists (-)-epinephrine and (+)-epinephrine displayed also a biphasic character, indicating the presence of 54±3% and 63±7% high affinity binding sites respectively (table 6.1; fig. 6.4). The binding of ^{3}H-Rauwolscine to α_2-adrenoceptors was stereoselectively inhibited by the stereoisomers of epinephrine. In the presence of 5.10^{-4} M Gpp(NH)p the (-)-epinephrine inhibition curve became monophasic (Ki=0.46±0.03 μM, n=3, fig. 6.5).

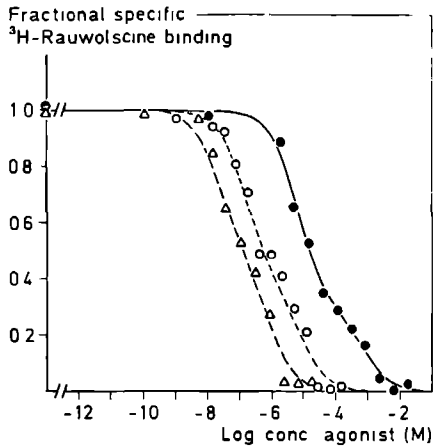

Fig. 6.4:
Inhibition of the specific ^{3}H-Rauwolscine binding in rat nasal mucosa membranes by the full agonists oxymetazoline (Δ), (-)-epinephrine (O) and (+)-epinehrine (●). All curves could be significantly better fitted according to a two binding sites model. Data shown are the means of triplicates from a representative experiment.

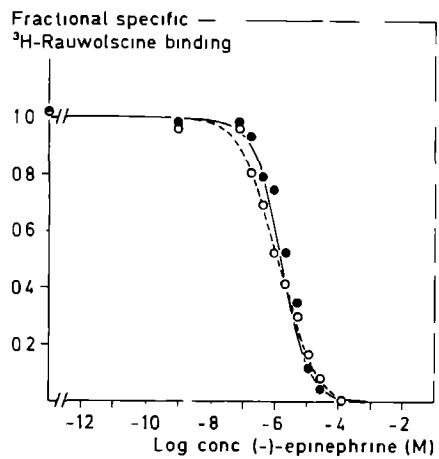

Fig. 6.5:
Inhibition of the specific ^{3}H-Rauwolscine binding in rat nasal mucosa membranes by the agonist (-)-epinephrine in the absence (O) and presence (●) of 5.10^{-4} M Gpp(NH)p. The inhibition curve in the presence of Gpp(NH)p could not be significantly better fitted according to a two binding sites model. Data shown are the means of triplicates from a representative experiment.

Table 6.1: Inhibition of α_2-adrenergic agonists and antagonists for ^{3}H-Rauwolscine (8 nM) binding to rat nasal mucosa membranes.

Agent	Ki (μM)	Ki (μM)	%RH	n
Antagonists				
yohimbine	0.037 ±0.010	-	-	3
phentolamine	0.013 ±0.006	-	-	4
prazosin	0.0012/0.0009	0.33/0.36	36/45	2
Agonists				
clonidine	0.033±0.009	-	-	3
oxymetazoline	0.006/0.012	0.62/0.44	52/56	2
(-)-epinephrine	0.11 ±0.03	2.12±0.26	54±3	3
(+)-epinephrine	2.5 ±0.6	125 ± 50	63±7	3

Further analysis of the ^{3}H-Rauwolscine binding to rat nasal mucosa indicated that in 7 out of 14 experiments the Scatchard plot pointed to an homogeneous class of binding sites (Kd=3.6±0.6 nM; Bmax=5.1±0.7 pmol/g). In the other experiments the Scatchard plot of the ^{3}H-Rauwolscine binding was curvilinear and the binding could be significantly better fitted according to a two binding sites model assuming high affinity binding sites with Kd_H=0.20±0.08 nM and Bmax=0.5±0.1 pmol/g tissue and low affinity binding sites with Kd_L=17±6 nM and Bmax=11±5 pmol/g.

The non-specific binding was identical, when determined in the presence of 12 μM phentolamine or 1 μM (-)-epinephrine. In the first experiments (n=14) the non-specific binding values were determined with phentolamine for two concentrations of radioligand. In 7 additional experiments, the non-specific binding values were determined for every concentration of the radioligand. In all these experiments the non-specific binding appeared to be non-linear (fig. 6.6). Neglecting the non-linearity of the non-specific binding, the specific ^{3}H-Rauwolscine binding was significantly better fitted according to a two binding sites model (fig. 6.6 A and 6.6 C) in 2 out of 7 experiments. However, the specific binding could adequately be fitted according to a one binding site model

if the non-specific binding values, fitted according to a saturation curve, were subtracted from the total binding in these 2 experiments (fig. 6.6 B and 6.6 D).

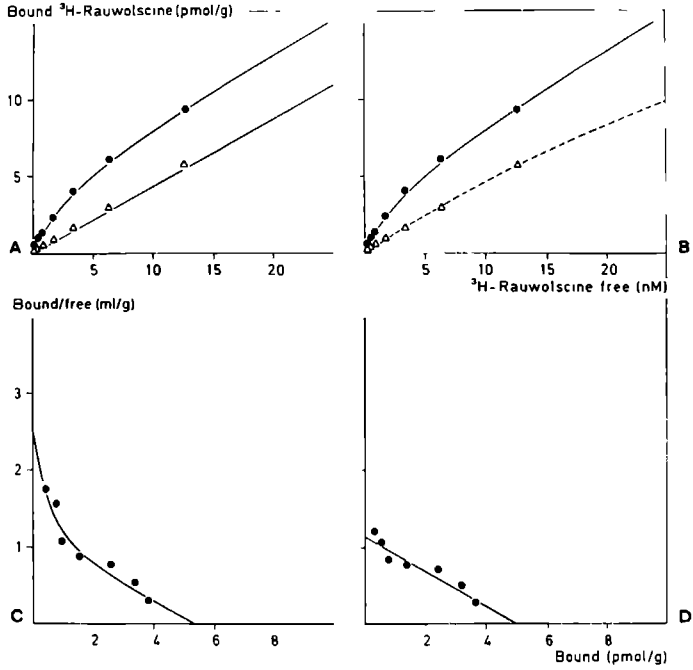

Fig. 6.6: Specific binding of ^{3}H-Rauwolscine to α_2-adrenoceptors in rat nasal mucosa membranes. Total binding (●) and non-specific binding (△), determined in the presence of 12 μM phentolamine. If in 2 out of 7 experiments the non-specific binding was fitted linearly (A), the specific binding (C; Scatchard plot) could be significantly better fitted according to a two binding sites model. If in these 2 experiments the non-specific binding was fitted according to a saturation curve (B), the specific binding (D, Scatchard plot) could adequately be fitted according to a one binding site model.

6.5. Discussion

Specific binding of ^{3}H-Rauwolscine to rat nasal mucosa was saturable and of high affinity. Careful analysis of the ^{3}H-Rauwolscine binding indicated that the binding occurs to an homogeneous class of binding sites with a Kd value of 3.6±0.6 nM and a receptor density of 5.1±0.7 pmol/g tissue or 204±28 fmol/mg protein in 7 out of 14 experiments. These Kd-values are in agreement with those of ^{3}H-Rauwolscine binding to other rat (Bylund, 1985; Adams et al., 1986; Pettibone et al, 1987) and human tissues (Muller and Noack, 1988). Surprisingly, in the other experiments the ^{3}H-Rauwolscine binding was significantly better fitted according to a two binding sites model, indicating high affinity binding sites with Kd_H=0.20±0.08 nM and Bmax=0.5±0.1 pmol/g tissue and low affinity binding sites with Kd_L=17±6 nM and Bmax=11±5 pmol/g. The total receptor density (11.5 pmol/g tissue), determined by a two binding sites analysis was higher than the density (5.1 pmol/g tissue) determined by a one binding site analysis. This discrepancy may be due to an overestimation of the number of binding sites in the analysis according to a two binding sites model and to scatter of the data points. A few studies reported curvilinear Scatchard plots with ^{3}H-Rauwolscine binding to other tissues (Diop et al., 1983; U'Prichard, 1984; Periyasamy and Somani, 1985; Convents et al., 1989a). The K_H and the K_L values determined in this study are in agreement with those reported for ^{3}H-Rauwolscine binding to rat and bovine brain cortex (Diop et al., 1983; U'Prichard, 1984) and human platelets (Periyasamy and Somani, 1985). Many interpretations of such binding data were given; binding to several α_2-adrenoceptor subclasses with different affinities, negative cooperative interactions among α_2-adrenergic binding sites (Periyasami and Somani, 1985), ^{3}H-Rauwolscine binding to serotonergic sites (Broadhurst et al., 1988; Convents et al., 1989b), or an inappropriate definition of the non-specific binding (Convents et al., 1989a). Differences in the non-specific binding determined in the presence of phentolamine or (-)-epinephrine have been reported (Convents et al., 1987; Convents et al., 1989a). In calf retina phentolamine should be used to define the non-specific binding since specific ^{3}H-Rauwolscine binding comprises

binding to α_2-adrenoceptors and non-adrenergic sites, when (-)-epinephrine is used for the determination of the non-specific binding (Convents et al., 1987). On the other hand, in human and rabbit cortical membranes (-)-epinephrine should be used to define the non-specific binding since specific ^{3}H-Rauwolscine binding comprises binding to α_2-adrenoceptors and non-receptor sites, when phentolamine is used for the determination of the non-specific binding (Convents et al., 1989b). In this study the non-specific binding was identical when determined in the presence of 12 μM phentolamine or 1 μM (-)-epinephrine. Non-linear non-specific binding has been reported in calf retina (Convents et al., 1987) and human and rabbit cortical membranes (Convents et al., 1989a), irrespective as to whether phentolamine or (-)-epinephrine were used to define non-specific binding. In this study, the non-specific binding appeared to be non-linear, when the non-specific binding values were determined for every concentration of the radioligand in 7 additional experiments. This non-linear non-specific binding may be due to filter binding since in most experiments non-specific binding consists of 85% and 50% filter binding at low and high concentrations radioligand, respectively. Neglecting the non-linearity of the non-specific binding the specific binding could adequately be fitted according to a one binding site model in 5 experiments, but could be significantly better fitted according to a two binding sites model in the other 2 experiments. If the non-linear, non-specific binding values for every concentration radioligand were subtracted from the total binding, the specific binding could adequately be fitted according to a one binding site model in these 2 experiments. This investigation of the non-specific binding suggested that the appearance of two ^{3}H-Rauwolscine binding sites are probably an artefact of an inappropriate definition of the specific binding due to non-linear non-specific binding. The irregular ^{3}H-Rauwolscine filter binding may be responsible for the non-linear, non-specific binding.

The kinetic experiments and competition experiments with yohimbine in our study also pointed to the presence of an homogeneous class of ^{3}H-Rauwolscine binding sites.

Apart from the artificial appearance of a second ^{3}H-Rauwolscine binding site, the population of α_2-adrenoceptors as labelled by ^{3}H-Rauwolscine

has been divided into subtypes on the basis of the affinity for prazosin (Bylund, 1985; Nahorski et al., 1985). The inhibition curve of ^{3}H-Rauwolscine with prazosin suggested the presence of α_2-adrenoceptor subclasses in rat nasal mucosa. The Ki-values of the high and low affinity binding sites were in agreement with those observed in rat lung (Bylund, 1988), rat submandibular gland (Bylund, 1985; Bylund, 1988) and human brain (Petrash and Bylund, 1986). In this study the Ki-value of prazosin for the high affinity sites was of the same magnitude as the Kd-value for ^{3}H-Prazosin binding to the α_1-adrenoceptors in rat nasal mucosa (van Megen et al., 1989). However, the α_1-adrenoceptor density (0.36 pmol/g tissue) in rat nasal mucosa determined with ^{3}H-Prazosin is lower than the receptor density expected from these experiments (approximately 2.9 pmol/g tissue). Other studies demonstrated an homogeneous class of α_2-adrenoceptors with low affinities for prazosin (Neylon and Summers, 1985; Hamilton et al., 1988; Convents et al., 1989a).

All full agonist inhibition curves of ^{3}H-Rauwolscine binding displayed high and low affinity agonist states. The Ki-value of the high affinity state of (-)-epinephrine is somewhat higher than described in other tissues, but a wide range of Ki-values has been reported (Michel et al., 1980; Brodde et al., 1982; Murphy and Bylund, 1988; Convents et al., 1989a). It has been accepted that the high affinity complex consists of agonist, receptor and G-protein (Michel et al., 1980; U'Prichard, 1984). In the presence of 5.10^{-4} M Gpp(NH)p a monophasic (-)-epinephrine inhibition curve was obtained. The Ki-value was in between the high and the low affinity Ki-values, probably due to a residual population of high affinity states, suggesting an incomplete conversion of the high into the low affinity binding sites. Complete conversion has neither been demonstrated in rat renal cortex (Snavely and Insel, 1982), human platelets (Schloos et al., 1987) and calf retina (Convents et al., 1989a). The Ki-values of oxymetazoline were in agreement with those determined in other studies (Rouot et al., 1982; Murphy and Bylund, 1988). Oxymetazoline and clonidine are clinically used nasal decongestants and have been reported to act mainly on the capacitance vessels, regulating the blood volume, but also on the resistance vessels decreasing the blood flow (Andersson and Bende, 1984; Ichimura and Chow,

1988). Physiological experiments in animals have also demonstrated the presence of α_2-adrenoceptors in nasal blood vessels (Ichimura and Jackson, 1984; Berridge and Roach, 1986). Autoradiographic experiments will be needed to localize these ^{3}H-Rauwolscine binding sites.

In the present study a radioreceptor assay has been developed for the demonstration and characterization of α_2-adrenoceptors in rat nasal mucosa. This assay will be applied to human nasal mucosa in order to investigate the supposed changes in the distribution pattern of receptors in nasal hyperreactivity.

Acknowledgement

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CHARACTERISTICS OF NON-ALLERGIC AND ALLERGIC PATIENTS

7.1. Introduction

Nasal hyperreactivity can best be defined as an increased response of a nasal effector system to a stimulus (Widdicombe, 1983). In specific nasal hyperreactivity or allergic rhinitis the specific stimuli are allergens (pollen, microfungi, house dust mites, animal products -dander and urine). The nasal symptoms (responses) are sneezing, hypersecretion and nasal obstruction (Mygind, 1978; Passali, 1983; Norman, 1985; Mygind, 1986). These symptoms in nasal allergy may be due to changes in the pharmacological characteristics of neuroreceptors (Mygind, 1982). The clinical picture and classification of patients is essential in the investigation of this hypothesis by radioligand receptor binding and in vitro autoradiographic studies of human nasal mucosa. In this study patients were classified into non-allergic and allergic individuals on the basis of clinical parameters. The heterogeneous non-allergic group was subdivided into control, chronic sinusitis and vasomotor rhinitis patients.

7.2. Materials and methods

Patients, from whom we received a biopsy, were characterized on the basis of the following parameters (according to Mygind, 1978): nasal symptoms, family history, sinus X-rays, serum IgE, blood and nasal eosinophilia, RAST and skin-tests. Patients were classified into a non-allergic and allergic group. The non-allergic group was subdivided into control individuals, chronic sinusitis and vasomotor rhinitis individuals.
The control group consisted of patients with a history of nasal trauma, deviations of the septum and cosmetic problems and were characterized by low serum IgE values (<90 U/ml), a low eosinophil count in the blood (<225.10^6 cells/ml) or in the nose (<2%) and negative RAST and skin-

tests. The sinus X-rays were normal.
The sinus X-rays of the chronic sinusitis patients demonstrated a mucosal change but other characteristics were similar to the control group.
The pathophysiology of the vasomotor rhinitis group resembles the pathophysiology of the allergic group, but an allergic component can be excluded. All these patients had nasal obstruction and the other characteristics were similar to the control group.
Allergic rhinitis was diagnosed on high IgE values (>150 U/ml), an elevated eosinophil count in the blood (>320.10^6 cells/ml) and/or in the nasal mucosa (>5%) and a positive RAST and/or skin-test for house dust mites, pollen and/or dander.

7.3. Results and discussion

Patients were characterized on the basis of the parameters described above, and classified into a non-allergic and allergic group (table 7.1 and 7.2). Furthermore, the heterogeneous non-allergic group was subdivided into controls, chronic sinusitis and vasomotor rhinitis patients. The patients should be diagnosed on the basis of the combination of parameters, since not all parameters of allergic patients differed from those of controls. Not all parameters could be obtained from all the patients, especially in the non-allergic group where RAST and skin-tests were rarely performed. Fourty-one out of 221 patients, from whom we received a biopsy, were left out of the study, because no clear distinction between allergic and non-allergic could be made. Another group of 114 patients was not involved in this study because of the limited amount of tissue received.
The clinical parameters of serum IgE, blood and nasal eosinophils were sometimes determined on two different points of time and the values were within the same order of magnitude with 85%, 76% and 66% reliability respectively. Mygind (1978) reported a 71%, 82% and 88% reliability of an increased number of eosinophils in the nose, an increased serum IgE content and a positive skin-test respectively in allergic patients.
This study comprises 26 allergic and 40 non-allergic individuals. The control group consisted of 28 individuals with a mean age of 31±11 years,

13 males and 15 females, 16 smokers and 5 individuals with medication. The chronic sinusitis group consisted of 9 patients with a mean age of 44±15 years, 5 males and 4 females, 2 smokers and 4 patients with medication. The vasomotor rhinitis group was a small group with 3 patients (mean age 37±24 years), 1 male and 2 females, 2 smokers and 2 patients with medication. The allergic group consisted of 26 patients with a mean age of 24±9 years, 16 males and 10 females, 4 smokers. Fifty percent of the patients were on medication and 38% also had asthma. The skin-test showed a positive reaction in 20 of the 26 patients with pollen (n=18), house dust mites (n=17) and/or dander (n=18). The RAST was positive in 5 out of 26 patients with pollen (n=4), house dust mites (n=5) and/or dander (n=2). The RAST and the skin-test both indicated a positive reaction in 3 out of 3 patients. None of the allergic patients demonstrated an acute clinical manifestation of allergic rhinitis.

In conclusion, only 30% of the biopsies could be used for biochemical studies since a clear distinction could not always be made between allergic or non-allergic patients and/or because of the limited amount of tissue.

Table 7.1: Characteristics of non-allergic patients.

Biopsy	birth-date	m/f	operation	date	medication	smoker	asthma
1	621008	f	turb	840807	Vi, T	2 c/d	no
2	650308	f	nr	840813	none	10 c/d	no
3	590907	f	turb str	840910	none	3 c/d	no
4	541011	f	sr	841102	none	10 c/d	no
5	401017	m	sr	841121	none	11 c/d	no
6	460215	m	revision	841210	Te	10 c/d	no
7	450506	f	sr	841212	Bi, Pa	25 c/d	no
8	510625	f	sr	850125	none	no	no
9	220611	m	polyp	850124	Be	no	yes
10	670905	m	turb str	850308	none	no	no
11	420518	f	turb	851107	Be, Ot	no	no
12	610612	f	turb str	850918	Be	no	yes
13	420401	f	turb str	851002	Be	no	no
14	310101	m	turb str	851118	none	no	child
15	560906	m	sr	860313	none	3 c/d	child
16	540212	f	sr	860405	none	25 c/d	no
17	580710	m	sr	860502	none	20 c/d	no
18	390903	m	nr	860605	none	8 c/d	no
19	630715	m	nr	860625	none	8 c/d	no
20	540817	m	sr	860627	none	15 c/d	no
21	640920	f	sr	860704	none	no	no
22	650426	m	sr	860711	none	no	no
23	650426	m	sr	860711	none	no	no
24	510827	m	sr	861014	none	no	no
25	240611	f	sr	861112	Au	no	no
26	230808	m	sr	870114	none	7 c/d	no
27	521222	f	sr	870206	Lo	no	no
28	550416	f	sr	870213	none	no	no
29	670730	f	turb str	870316	none	5 c/d	no
30	420725	f	sr	870431	Syn	25 c/d	no
31	721215	m	turb str	870702	none	no	no
32	690721	f	sr	870708	none	10 c/d	no
33	430920	f	polyp	870820	none	no	no
34	580414	m	sr	870824	none	10 c/d	no
35	620203	f	sr	871005	none	no	no
36	540405	m	sr	871211	none	no	no
37	470726	m	turb str	880208	none	8 c/d	no
38	690621	f	sr	870728	none	no	no
39	401211	m	sr	870728	none	25 c/d	no
40	220309	f	polyp	881004	La	no	no

Au=Augmentine, Be=Beconase, Bi=Bisolvon, c=control, c/d=cigarettes/day, child=asthma in childhood, Da=dander, Eo=Eosinophils, HM=house dust mites, La=Lanoxin, Lo=Lomudal, neg=negative, ND=not determined, nr=nose resection, Ob=Obstruction, Ot=Otrivin, Pa=paracetamol, Po=pollen, polyp=polypectomy, pos=positive, Rh=Rhinorhoe, rv=rhinitis vasomotoria, s=chronic sinusitis, s(p)=chronic sinusitis with polyps, Sn=sneezing, sr=septum resection, Syn=Syntaris, T=Triludan, Te=Tetracycline, Tr=trauma, turb=turbinectomy, turb str= stripping turbinates,Vi=Vibramycine.

Table 7.1: continued

Biopsy	date param.	IgE (U/ml)	Eo blood ($x10^6$ c/ml)	Eo nose (%)	septum-deviation	X-ray (date)
1	840821	22	110	0	neg	neg(831117)
2	ND	ND	ND	ND	pos	ND
3	831086	7	120	0	neg	neg(831026)
4	841120	6	65	0	neg	neg(840315)
5	861210	ND	70	70	neg	pos(841108)
6	821216	ND	70	0	neg	pos(860312)
7	ND	ND	ND	ND	neg	pos(841129)
8	850219	28	50	0	neg	pos(841024)
9	710514	ND	226	ND	neg	pos(830314)
10	841019	10	90	3	pos	neg(840000)
11	850722	49	120	0	neg	neg(850227)
12	850930	96	180	0	neg	ND
13	850129	27	125	0	neg	pos(850129)
14	860425	14	150	0	neg	pos(850925)
15	ND	ND	ND	ND	neg	ND
16	ND	ND	ND	ND	pos	ND
17	860623	54	265	5	pos	neg(860110)
18	860610	34	220	5	pos	neg(850827)
19	ND	ND	ND	ND	pos	ND
20	860402	44	80	0	pos	neg(860404)
21	861007	10	80	0	pos	neg(801112)
22	ND	ND	ND	ND	pos	ND
23	851223	6	165	0	pos	neg(860204)
24	860709	149	45	0	pos	neg(860709)
25	ND	ND	ND	ND	neg	pos(861103)
26	790514	73	273	25	pos	ND
27	861106	32	110	0	pos	neg(861204)
28	860829	15	ND	ND	neg	neg(860926)
29	860910	102	300	5	neg	ND
30	870202	86	12	2	pos	neg(870223)
31	ND	ND	ND	ND	neg	pos(831101)
32	871117	2	60	0	pos	neg(870321)
33	870901	51	80	0	neg	pos
34	870910	119	215	0	pos	ND
35	ND	ND	ND	ND	pos	ND
36	880120	10	50	0	neg	neg(871026)
37	870313	23	135	0	neg	neg(870623)
38	ND	ND	ND	ND	pos	ND
39	ND	ND	ND	ND	neg	neg(880330)
40	ND	ND	ND	ND	neg	neg(780720)

Table 7.1: continued

Biopsy	Skin-test	RAST-test	Sn	Rh	Ob	Tr	Diagnosis
1	ND	neg (831222)	neg	neg	pos	neg	rv
2	ND	ND	ND	ND	pos	pos	c
3	ND	ND	neg	pos	pos	neg	c
4	ND	ND	ND	ND	pos	neg	c
5	ND	ND	pos	pos	pos	neg	s
6	ND	ND	ND	pos	pos	neg	s
7	ND	ND	ND	neg	pos	neg	c
8	ND	ND	ND	neg	pos	neg	s
9	neg (710514)	ND	ND	neg	pos	neg	s
10	neg (841019)	ND	ND	pos	pos	neg	c
11	ND	ND	pos	pos	pos	neg	c
12	neg (850000)	ND	pos	pos	pos	pos	rv
13	neg (860515)	ND	pos	pos	pos	neg	s
14	ND	ND	ND	pos	pos	neg	s
15	ND	ND	ND	neg	pos	neg	c
16	ND	ND	neg	neg	pos	neg	c
17	ND	ND	neg	neg	pos	neg	c
18	ND	ND	pos	pos	pos	neg	c
19	ND	ND	ND	ND	pos	neg	c
20	ND	ND	neg	pos	pos	neg	c
21	ND	ND	ND	pos	pos	neg	c
22	ND	ND	neg	pos	pos	pos	c
23	ND	ND	pos	neg	pos	neg	c
24	ND	ND	neg	neg	pos	neg	c
25	ND	ND	ND	pos	ND	neg	s
26	neg (790514)	ND	pos	pos	pos	neg	rv
27	ND	ND	neg	neg	pos	neg	c
28	ND	ND	ND	neg	pos	neg	c
29	ND	neg (860910)	ND	pos	pos	neg	c
30	ND	ND	pos	neg	pos	neg	c
31	ND	ND	neg	neg	pos	neg	s(p)
32	ND	ND	pos	pos	pos	neg	c
33	neg (630125)	ND	neg	pos	pos	pos	s(p)
34	ND	ND	ND	neg	ND	neg	c
35	ND	ND	ND	neg	pos	pos	c
36	Po, Da (540410)	ND	ND	pos	pos	neg	c
37	ND	neg (870602)	ND	pos	pos	neg	c
38	ND	ND	neg	neg	pos	neg	c
39	ND	ND	ND	pos	pos	neg	c
40	ND	ND	pos	pos	pos	neg	c

Table 7.2: Characteristics of allergic rhinitis patients.

Biopsy	birth-date	m/f	operation	date	medication	smoker	asthma
41	580928	m	sr	840809	none	no	no
42	641014	f	turb str	841008	Be	no	yes
43	650714	f	turb str	841210	none	ND	no
44	620708	f	sr	841210	none	25 c/d	yes
45	640716	m	sr	850103	Be	no	yes
46	590619	m	sr	850218	Ve	no	no
47	520807	m	nr	850219	none	3 c/d	child
48	640110	m	sr	850304	Me	no	no
49	730809	m	turb str	850509	Ro, Pe	no	no
50	610711	m	turb str	850730	Be	no	yes
51	570703	f	sr	860223	none	no	no
52	631106	f	sr	860415	Be, Lo	no	yes
53	611124	f	sr	860623	Be, Meth	25 c/d	yes
54	690427	m	sr	860702	none	no	no
55	641203	m	turb str	860908	none	no	no
56	691031	f	turb str	861031	Ti	no	no
57	440201	f	turb str	861219	none	no	no
58	711220	m	turb str	870105	none	no	no
59	370114	f	turb str	870227	Be	no	no
60	580101	m	turb str	870728	The	no	child
61	651116	m	turb str	871112	Ve	no	yes
62	700107	m	sr	871228	Syn	no	yes
63	621201	m	sr	880114	none	no	yes
64	640315	m	sr	880115	none	25 c/d	no
65	590730	f	turb	880307	none	no	no
66	800128	m	turb	880915	Lo, Ve	no	yes

ar=allergic rhinitis, Be=Beconase, c=control, c/d=cigarettes/day, child= asthma in childhood, Da=dander, Eo=Eosinophils, HM=house dust mites, Lo=Lomudal, Me=Methyrit, Meth=Methadon, neg=negative, ND=not determined, nr=nose resection, Ob=Obstruction, param.=parameters, Pe=Penicillin, Po=pollen, polyp=polypectomy, pos=positive, Rh=Rhinorhoe, Ro=Ronnisol, s=chronic sinusitis, s(p)=chronic sinusitis with polyps, Sn=sneezing, sr=septum resection, Syn=Syntaris, The=Theoline, Ti=Tinset, Tr=trauma, turb=turbinectomy, turb str= stripping turbinates, Ve=Ventolin, vr=vasomotor rhinitis.

Table 7.2: continued

Biopsy	date param.	IgE (U/ml)	Eo blood ($x10^6$ c/ml)	Eo nose (%)	septum-deviation	X-ray (date)
41	830419	ND	34	0	neg	neg(830503)
42	841023	155	60	2	pos	neg(841023)
43	841031	ND	410	30	ND	pos
44	830118	2090	320	50	neg	neg(830201)
45	850118	2880	255	0	neg	ND
46	841011	566	405	90	neg	neg(841012)
47	840727	ND	1320	50	neg	ND
48	850329	1235	480	0	neg	ND
49	ND	ND	ND	ND	neg	neg(840501)
50	ND	ND	ND	ND	neg	cele(850730)
51	860317	468	100	0	neg	neg(860102)
52	ND	ND	ND	ND	neg	ND
53	860702	107	105	0	neg	neg(860424)
54	861208	940	575	10	neg	ND
55	860917	142	165	5	neg	neg(860624)
56	ND	ND	ND	ND	neg	neg(861006)
57	860618	578	465	5	neg	neg(860805)
58	860120	226	685	5	neg	pos(860217)
59	870515	168	170	10	neg	neg(861008)
60	ND	ND	ND	ND	ND	ND
61	871123	2120	330	2	neg	neg(870617)
62	870224	4650	ND	ND	pos	pos(870702)
63	880127	1110	286	0	neg	ND
64	880603	720	435	60	pos	neg(880208)
65	880324	440	200	15	neg	ND
66	890109	ND	1900	2	neg	neg(880808)

Table 7.2: continued

Biopsy	Skin-test	RAST-test	Sn	Rh	Ob	Tr	Diagnosis
41	pos Po, HM, Da (830607)	ND	pos	pos	pos	neg	ar
42	pos Po, Da (860403)	ND	pos	pos	pos	neg	ar
43	pos Po, HM (841013)	pos Po, HM	pos	ND	ND	neg	ar
44	pos Po, HM, Da (840619)	ND	pos	pos	pos	neg	ar
45	pos Po, HM, Da (850228)	ND	pos	pos	pos	neg	ar
46	pos Po, HM, Da (841113)	ND	pos	pos	pos	neg	ar
47	ND	ND	neg	pos	pos	neg	ar
48	pos Po, HM, Da (840000)	ND	pos	pos	pos	neg	ar
49	ND	Po, HM, Da	pos	neg	pos	neg	ar
50	pos Po, HM, Da (840000)	ND	pos	neg	pos	pos	ar
51	pos Po, HM, Da (820102)	ND	pos	pos	pos	neg	ar
52	ND	HM(860000)	ND	pos	pos	neg	ar
53	ND	ND	neg	pos	pos	neg	ar
54	pos Po, HM, Da (870217)	ND	pos	pos	pos	neg	ar
55	pos Po, HM, Da (860624)	Po, HM	ND	pos	pos	neg	ar
56	pos HM (860000)	ND	pos	pos	pos	neg	ar
57	pos Po, Da (860805)	ND	pos	neg	pos	neg	ar
58	ND	ND	pos	pos	pos	neg	ar
59	pos Po, Da (620309)	ND	neg	neg	pos	neg	ar
60	pos Po, HM, Da (860000)	ND	neg	neg	pos	neg	ar
61	ND	ND	ND	pos	pos	neg	ar
62	pos Po, HM, Da (870702)	Po, HM, Da	pos	pos	pos	neg	ar
63	pos Po, HM, Da (830000)	ND	pos	pos	pos	neg	ar
64	pos Po, HM, Da (861202)	ND	pos	pos	pos	neg	ar
65	pos HM (870000)	ND	pos	neg	pos	neg	ar
66	pos Po, HM, Da (880000)	ND	neg	neg	pos	neg	ar

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HISTOLOGICAL CHANGES IN THE NASAL MUCOSA OF ALLERGIC PATIENTS IN COMPARISON WITH NON-ALLERGIC PATIENTS.

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8.1. Abstract

A histological study of the nasal mucosa of non-allergic patients and allergic patients (allergic rhinitis) was performed. The non-allergic group was subdivided into control and chronic sinusitis patients. Specimens were embedded in glycolmethacrylate, stained with Toluidine blue and Alcian blue/Periodic acid Schiff and examined by light microscopy. In most specimens, various tissue components were present; epithelium and the lamina propria with glands and blood vessels. The AB/PAS staining indicated the presence of neutral as well as acidic glycoproteins in goblet cells and in the tubulo-alveolar glands. In contrast with the control individuals, chronic sinusitis patients showed granulocytes in the respiratory epithelium suggesting infection. In specimens of patients with allergic rhinitis basophilic cells were observed in the epithelium, whereas no basophilic cells could be observed in control individuals. More basophilic cells were observed in the lamina propria of allergic patients in comparison with controls.

8.2. Introduction

In the last decade the structure of the nasal mucosa from both control individuals and from patients with allergic rhinitis has been studied with light, scanning and electron microscopy (Jahnke, 1972; Mygind et al., 1974; Tos and Mogensen, 1977; Okuda et al, 1983; Kawabori et al.,

1983). Histological changes in epithelium, basal membrane and glands have been observed in allergic patients. Most histological studies were performed on nasal scrapings or blown secretion and only a few studies deal with biopsies from the nasal mucosa. In this study we present a blind histological investigation of plastic embedded biopsies of nasal mucosa from controls, from patients with chronic sinusitis and from patients with allergic rhinitis.

8.3. Materials and methods

Biopsies of human nasal mucosa from behind the inferior turbinate were obtained during sinus and septal surgery. Nasal allergy or chronic sinusitis was diagnosed on the basis of the following parameters; nasal symptoms, family history, sinus X-rays, blood and nasal eosinophils, serum IgE, RAST and skin-tests. Patients were classified into a non-allergic group, subdivided into control individuals and chronic sinusitis patients, and an allergic (allergic rhinitis) group. The study comprises 12 control patients (6 males and 6 females) with ages ranging from 20 to 50 years (mean age 34.5 years), 2 chronic sinusitis patients (1 male and 1 female, 17 and 47 years respectively) and 7 patients with allergic rhinitis (4 males and 3 females) ranging from 18 to 52 years (mean age 29 years). All allergic patients had a positive RAST and/or skin-test for house dust mites, whilst 4 patients had a positive test for pollen. None of the allergic patients showed an acute manifestation of allergic rhinitis.

The biopsies were immediately washed in 0.9% NaCl and fixed in 2.5% glutardialdehyde in 0.1 M phosphate buffer (pH 7.0) for 24 hours at 4°C. Specimens with bone were decalcified in 10% EDTA (pH 7.3) for 14 days. For embedding in glycolmethacrylate (GMA, JB-4 embedding kit, Polysciences Inc., Warrington PA, USA), the specimens were dehydrated in ethanol (from 70 to 100%) and impregnated with catalysed solution A (100 ml solution A with 0.9 g catalyst) overnight at 4°C. Subsequently, the specimens were placed in moulding cups and embedded in a mixture of 25 ml catalysed solution A and 0.9 ml solution B in a vacuum for 15-30 min at 4°C to avoid air interference with the polymerization process. The

plastic moulding cups were sealed with melted paraffin. Polymerization was continued at 0°C for 30 min and thereafter at 25°C for 60 min. The blocks were stored at 4°C.

JB-4 sections (2 µm), made with a glass knife, were floated on distilled water, containing 2-4 drops NH_4OH per 200 ml. The sections were picked up on glass slides and the slides were slightly warmed until wrinkles disappeared. The sections were stained with Toluidine blue or Alcian blue/Periodic acid Schiff and mounted in DPX-xylol.

Staining procedures.

Toluidine blue (TB)

Sections were stained in 2.5% TB (C.I. 52040) in 2.5% anhydrous sodiumcarbonate for 2 min, rinsed in distilled water until the excess of stain is removed and dried in air.

Alcian blue - Periodic acid Schiff (AB-PAS)

Sections were stained in AB solution (1% AB 8GX, C.I.74240) in 3% acetic acid, pH 2.6) for 90 min and rinsed in distilled water. Subsequently, the specimens were treated with 1% periodic acid for 15 min, rinsed in distilled water for 5 min and stained in Schiff's reagent for 60 min. Schiff's reagent was prepared by boiling distilled water, followed by the addition of 1% basic fuchsin (C.I. 42500). The solution was cooled to 50°C, filtered and 10% HCl (1N) was added. The solution was then cooled to 25°C, before 1% sodiummetabisulphite was added. After 1 day storage in the dark, the light brown solution was bleached with 2% bone charcoal and filtered. After staining in Schiff's reagent, the sections were rinsed 3 times for 10 min in fresh sulphurous acid (1.2% sodiummetabisulphite in 0.05 N HCl), repeated rinsing in tap water for 10 min and stained in Mayer's Hemalum for 10 min, followed by rinsing in tap water and dried in air.

8.4. Results

Controls

Most specimens (8 out of 12) contained pseudostratified ciliated

epithelium, whereas other specimens contained stratified respiratory epithelium (table 8.1). In 5 specimens squamous epithelium was present in some areas. The epithelium is resting on a basement membrane, which consists of a PAS-positive membrane with underneath a connective tissue membrane. In the pseudostratified columnar respiratory epithelium of most specimens, 4 cell types could be distinguished; basal cells, ciliated and non-ciliated columnar cells and goblet cells. The goblet cells stained with PAS and/or AB, indicating the presence of neutral and/or acidic glycoproteins. Hyperplasia, transudate and intra-epithelial glands were only occasionally observed. Infiltrating cells were not present in the epithelium.

The glandular tissue in the lamina propria, is of the tubulo-alveolar type. Acini are scattered throughout the respiratory region, surrounded by connective tissue, and drain by small unbranched excretory ducts. The glands stained with PAS and/or AB. Blood vessels were present in biopsies of human nasal mucosa; the sinusoids, large venous erectile tissue, were only present in a few specimens (table 8.2). Infiltrating cells, such as granulocytes, plasma cells and basophilic cells (PAS-positive and TB-positive) were present in the lamina propria.

Chronic sinusitis

The histological structure of the nasal mucosa of 2 patients with chronic sinusitis was comparable with the structure of controls. Many granulocytes were present in the respiratory epithelium of 1 patient, whereas respiratory epithelium could not be observed in the other patient (table 8.1, table 8.2).

Allergic rhinitis

Generally, the histological structure of the nasal mucosa of 7 patients with allergic rhinitis was comparable with the structure of the mucosa of the control individuals. However, basophilic cells (mast cells) were observed in the epithelium of the nasal mucosa of allergic patients, whereas none were observed in the epithelium of the controls. The number of basophilic cells in the lamina propria was also increased in allergic patients in comparison with controls.

Table 8.1: Histology of the epithelium of nasal biopsies of non-allergic (control individuals, chronic sinusitis) and allergic rhinitis patients.

nr.	m/f	Stratified	Squamous	GC	CNC	BC	BM	HH	INF	TR
Control individuals										
16	f	stratified	O	NO	NO	NO	O	NO	NO	NO
17	m	pseudostr.	NO	O	O	O	O	O	NO	NO
18	m	stratified	O	NO	NO	NO	NO	NO	NO	NO
19	m	stratified	O	O	O	O	O	NO	NO	NO
24	m	pseudostr.	NO	O	O	O	O	NO	NO	NO
28	f	pseudostr.	O	O	O	O	O	O	NO	NO
29	f	pseudostr.	NO	O	O	O	O	O	NO	O
30	f	pseudostr.	NO	NO	NO	NO	NO	NO	NO	NO
32	f	pseudostr.	NO	O	O	O	O	NO	NO	NO
35	f	pseudostr.	NO	O	O	O	O	NO	NO	NO
37	m	stratified	NO	O*	O	O	O	O	NO	O
40	m	pseudostr.	O	O	O	O	O	NO	NO	NO
Chronic sinusitis										
13	f	stratified	O	NO	NO	NO	O	NO	NO	NO
31	m	pseudostr.	NO	O	O	O	O	NO	GR	NO
Allergic rhinitis										
56	f	pseudostr.	O	O	O	O	O	O	MC,GR	O
58	m	pseudostr.	NO	O	O	O	O	NO	MC	NO
59	f	pseudostr.	O	O	O	O	O	NO	MC	NO
60	m	stratified	NO	O*	O	O	O	O	MC	NO
63	m	pseudostr.	NO	O	O	O	O	NO	MC	NO
64	m	pseudostr.	O	NO	O	O	O	NO	MC	NO
65	f	stratified	O	O	O	O	O	O	MC	NO

BC=Basal cells, BM=Basal membrane, CNC=Ciliated and non-ciliated cells, GC=Goblet cells, GL=Glands, GR=Granulocytes, HH=Hyperplasia, INF=Infiltrate, MC=mast cells, NO=Not observed, O=Observed, TR=Transudate, *=forming intra-epithelial glands.

Table 8.2: Histology of the lamina propria of nasal biopsies of non-allergic (control individuals, chronic sinusitis) and allergic rhinitis patients.

Nr.	Infiltrate	Glands (glycoproteins)		Blood vessels	
		PAS	AB	Sinusoids	Others
Control individuals					
16	MC, GR	POS	ND	O	O
17	MC, PC	POS	ND	MANY	O
18	FEW MC	POS	ND	NO	O
19	FEW MC	POS	ND	NO	O
24	FEW MC,	POS	POS AND NEG	O	MANY
28	MC, PC	POS	POS AND NEG	O	O
29	MC, GR	POS	POS AND NEG	O	O
30	MC, GR	POS	POS AND NEG	NO	O
32	MC, GR, PC	POS	POS AND NEG	O	O
35	MC, GR	POS	POS AND NEG	O	O
37	MC	NO	NO	NO	NO
40	MC, GR	POS	POS AND NEG	NO	O
Chronic sinusitis					
13	FEW MC, GR, PC	POS	ND	NO	O
31	MC, PC	NO	NO	NO	O
Allergic rhinitis					
56	MANY MC, GR, PC	POS	POS AND NEG	NO	MANY
58	MANY MC, PC	POS	POS AND NEG	MANY	O
59	MANY MC, GR, PC	POS	POS AND NEG	O	O
60	MC, GR, PC	POS	POS AND NEG	O	O
63	MC, GR, PC	POS	POS AND NEG	NO	O
64	MC, GR, PC	POS	POS AND NEG	NO	O
65	MC, GR, PC	POS	POS AND NEG	O	O

AB=Alcian blue, GR=Granulocytes, MC=Mast cells, ND= Not determined, NO=Not observed, O=Observed, PAS=Periodic acid Schiff, PC=Plasma cells.

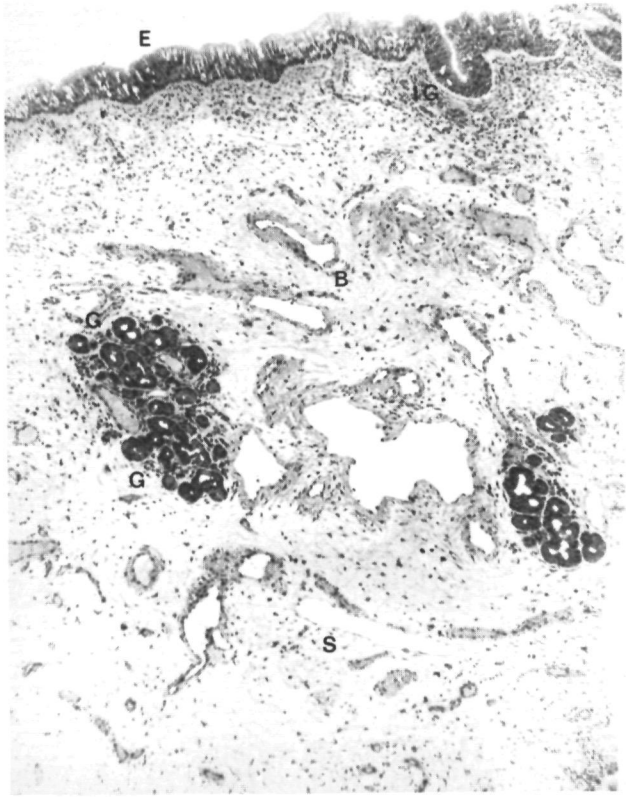

Fig 8.1: Micrograph of the human nasal mucosa, showing epithelium (E) forming intra-epithelial glands (IG), glands (G), blood vessels (B) and sinusoids (S). TB staining, x50.

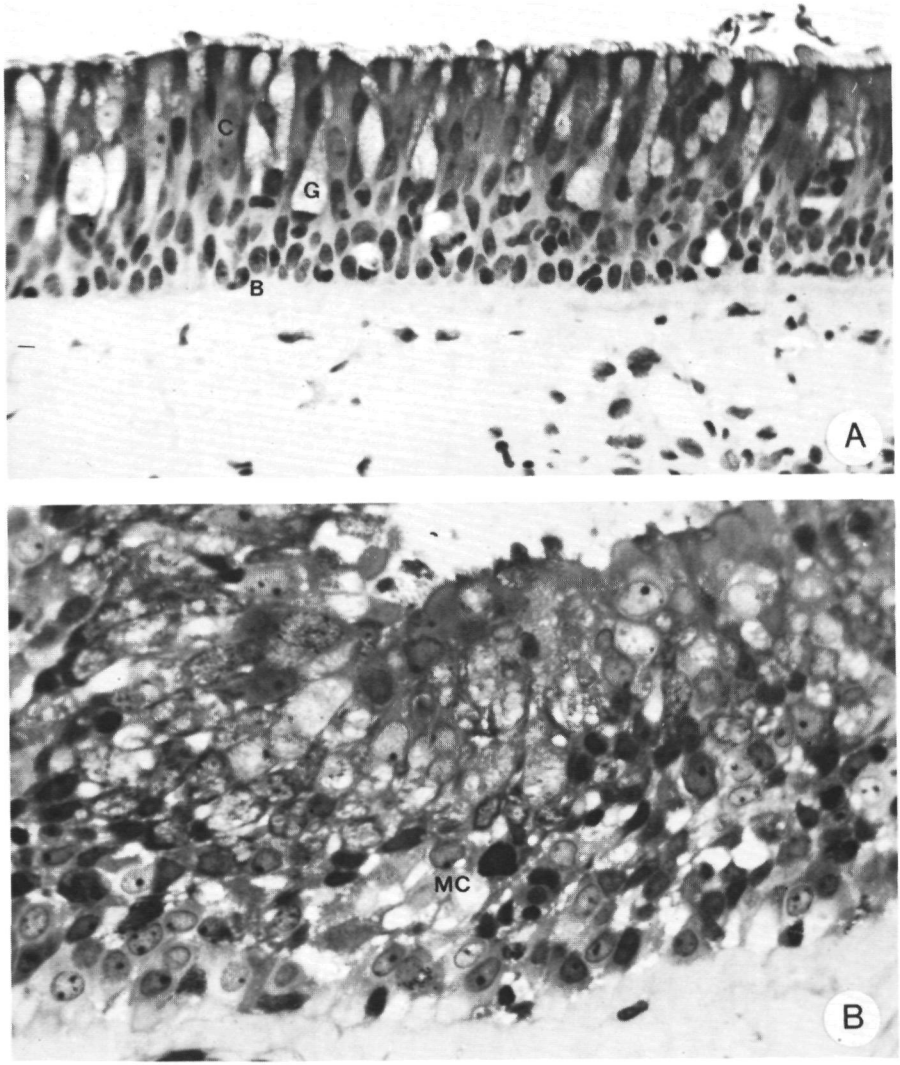

Fig. 8.2: Micrograph of the epithelium of the nasal mucosa of a control (A) and an allergic rhinitis (B) patient, showing basal cells (B), goblet cells (G) and ciliated cells (C). The mast cells (MC) in the epithelium are characteristic for the allergic patients. The hyperplasia of the epithelium occurs in allergic as well as control patients. TB staining, x500.

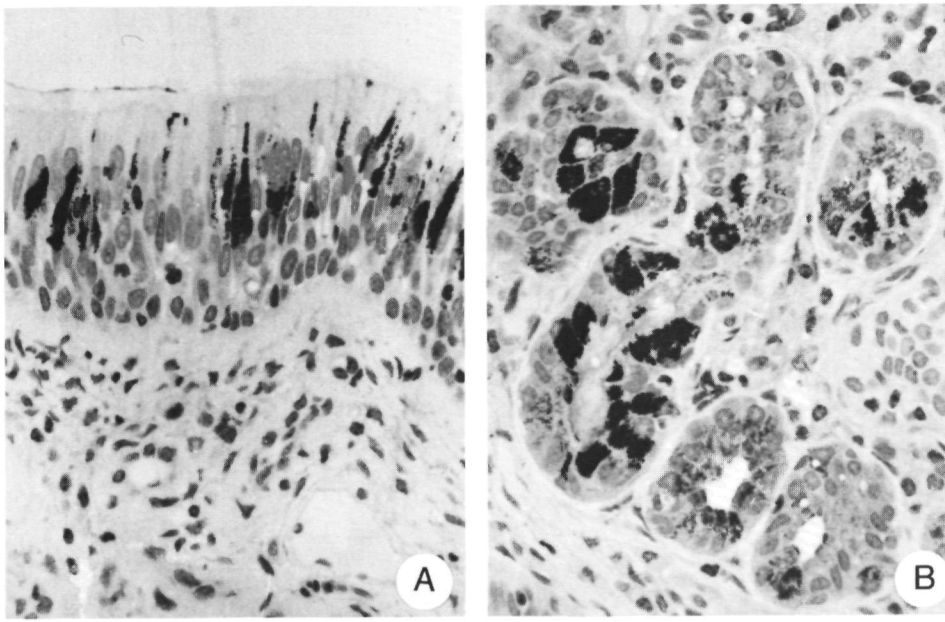

Fig. 8.3: Micrograph of the epithelium (A) and glands (B) in the nasal mucosa of a control patient, showing glycoproteins. AB/PAS staining, x500.

8.5. Discussion

Various tissue components were present in specimens of nasal mucosa of control, chronic sinusitis and allergic patients.

Most specimens of the controls contained pseudostratified respiratory epithelium, whereas other specimens contained stratified squamous epithelium. These observations are in agreement with other studies of human nasal mucosa (Mygind et al., 1974). In the pseudostratified respiratory epithelium, basal cells, ciliated and non-ciliated cells and goblet cells could be distinguished. These cell types have also been described in human (Rhodin, 1974; Mygind et al., 1982) and rat nasal mucosa (Klaassen et al., 1981). AB-PAS staining demonstrated red and blue secretory material in the goblet cells, indicating the presence of

neutral and/or acidic glycoproteins (Jones, 1978; Johannes and Klessen, 1984). AB- and PAS-positive goblet cells have also been observed in human nasal mucosa (Thaete et al., 1981).

The tubulo-alveolar glands in the lamina propria are scattered throughout the respiratory area and drain by small excretory ducts. These findings are in agreement with those observed in human nasal mucosa by Bojsen-Møller (1965) and by Tos and Mogensen (1977). Ultrastructural studies revealed that striated excretory ducts are absent in these glands (Jahnke, 1972). In all specimens the glandular tissue stained PAS-positive, indicating the presence of glycoproteins in human nasal glands. The PAS-positive glands were AB-negative or -positive, indicating the presence of neutral or neutral admixed with acidic glycoproteins in the acini. Glycoproteins are responsible for the viscosity and gelforming properties of the mucus. It has also been suggested that glycoproteins exert a protective role by trapping microorganisms (Clamp et al., 1978). No different glandular areas could be distinguished on the basis of the AB/PAS staining in human nasal mucosa. In rat nasal glands, three different areas could be distinguished on the basis of this staining for glycoproteins (Klaassen et al., 1981). Thaete et al. (1981) also observed PAS-negative and/or AB-positive glands in human nasal mucosa. The PAS-negative/AB-negative cells were also observed and described as serous cells, whereas the PAS-positive and AB-negative were described as mucous cells. However, a differentiation in mucous and serous cells cannot be made on account of their affinity for glycoprotein staining, because the secretory granules of both serous and mucous glands contain glycoproteins (Jones and Reid, 1978).

The histological structure of the nasal mucosa of the 2 patients with chronic sinusitis was comparable with the structure of the control individuals. Granulocytes were observed in the respiratory epithelium of 1 chronic sinusitis patient, whereas no respiratory epithelium could be observed in the other. Increased numbers of inflammatory cells have been described in other studies. Hypertrophy of the nasal glands could not be observed in this study but has been described elsewhere (Greenberg and Ainsworth, 1966; Okuda et al., 1983).

Specimens of patients with allergic rhinitis demonstrated basophilic

cells in the epithelium, whereas no basophilic cells could be observed in the controls. The number of basophils in the lamina propria was also increased in allergic patients in comparison with controls. Basophilic cells can be subdivided into blood basophilic leucocytes and tissue mast cells (Galli et al., 1984). Since the basophilic cells were present in biopsies of the nasal mucosa, mainly mast cells are present in the specimens. An increased number of mast cells in the epithelium and the lamina propria has been reported in histological studies of biopsies (Mygind et al., 1974; Tos and Mogensen, 1977; Okuda et al., 1983; Ohtsuka et al., 1985), in nasal scrapings (Liu, 1988) or nasal secretions (Ukai et al., 1986). Activation of mast cells leads to the release of mast cell mediators such as histamine from the granules (Robinson and Holgate, 1985; Wasserman, 1987). It has been suggested that the migration of basophilic cells to the surface is characteristic for allergy and that the total number and the histamine content of these cells are sufficient to produce the nasal manifestation of allergy (Okuda et al., 1983; Pipkorn et al., 1988).

Despite the increase in the number of basophilic cells in the epithelium and the lamina propria, no other histological changes have been observed in patients with allergic rhinitis in comparison with controls. Many histological changes in biopsies of the nasal mucosa from allergic patients have been reported; e.g. an increase in the number of goblet cells (Mygind et al., 1974; Okuda et al., 1983), hyperplasia of the epithelium (Tos and Mogensen, 1977), thickening of the basement membrane (Mygind et al., 1974), an increase in the number and density of the glands (Tos and Mogensen, 1977; Okuda et al., 1983), thickening of the wall of large and small blood vessels (Tos and Mogensen, 1977). Other studies have also reported no change in the number of goblet cells (Tos and Mogensen, 1977) or of the basement membrane (Mygind et al., 1974). These discrepancies of the observed histological structures may be due to differences in the site of the biopsy or differences in time of removal. In our study biopsies were taken from allergic patients with no acute clinical manifestation of the allergic rhinitis.

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ALTERATIONS OF MUSCARINIC ACETYLCHOLINE RECEPTORS IN THE NASAL MUCOSA OF ALLERGIC PATIENTS IN COMPARISON WITH NON-ALLERGIC INDIVIDUALS.

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9.1. Abstract

Cholinergic nasal hyperresponsiveness in nasal allergy may be due to changes of the characteristics in muscarinic cholinergic receptors. Radioligand receptor binding and in vitro autoradiographic studies of nasal mucosa in non-allergic and allergic patients were performed to investigate this hypothesis. The heterogeneous non-allergic group was subdivided into control, chronic sinusitis and vasomotor rhinitis patients. The ^{3}H-l-QNB binding to muscarinic receptors in human nasal mucosa membranes was saturable and of high affinity in all groups. No significant differences could be demonstrated between the subgroups of the non-allergic patients. In allergic patients the equilibrium dissociation constants and receptor densities were significantly decreased in comparison with those of non-allergic and control individuals. No differences in agonist binding or coupling of the muscarinic receptor to the effector system via the G-protein could be observed in allergic patients.
In vitro autoradiographic experiments demonstrated specific ^{3}H-l-QNB labelling of the glandular acini in non-allergic and allergic patients. No specific labelling could be observed in the epithelium, blood vessels or connective tissue.
In conclusion, the increased sensitivity and decreased receptor number may reflect the cholinergic induced hypersecretion in nasal allergy but are probably too small to explain the complex allergic reaction.

9.2. Introduction

Nasal hyperreactivity in allergic rhinitis may originate from an imbalance of the autonomic nerve regulation (Mygind, 1982). Cholinergic nasal hyperresponsiveness has been observed in allergic patients. Methacholine provocation resulted in a significant increased secretion, without changes in the nasal airway resistance in allergic patients in comparison with control individuals (Borum et al., 1983; Druce et al., 1985; Corrado et al., 1986; Devillier et al., 1988). Cholinergic hyperresponsiveness has been explained in terms of changes in characteristics of muscarinic cholinergic receptors in the lower airways of asthmatic patients (Raaijmakers et al., 1984; Nadel and Barnes, 1984; Barnes, 1986; Hahn, 1986). In contrast with the lower airways, only limited quantitative data (receptor densities) of muscarinic receptors in the human nasal mucosa are available (Ishibe et al., 1983; Konno et al., 1987). In this study radioligand receptor binding studies were performed with the antagonist ^{3}H-l-QNB to investigate muscarinic receptors in non-allergic and allergic patients in order to elucidate the supposed changes in the characteristics of these receptors in nasal hyperreactivity. Furthermore, the heterogeneous non-allergic group has been subdivided into control, chronic sinusitis and vasomotor rhinitis patients. The investigation of the muscarinic receptors includes not only the receptor density and sensitivity but also the agonist affinity states and the coupling of the muscarinic receptor to the effector system by G-proteins. In vitro autoradiographic experiments were performed to localize muscarinic receptors in the nasal mucosa of non-allergic and allergic patients.

9.3. Materials and methods

Materials

^{3}H-l-QNB (Quinuclidinylbenzilate)(spec. act. 33.1 Ci/mmol) was purchased from New England Nuclear, Doorn, The Netherlands. l-QNB was a gift from Dr. G. Lambrecht, Wolfgang Goethe University, Frankfurt, West-Germany. Methylfurtrethonium was synthesized in our laboratory according to known

procedures. HEPES, PMSF (phenylmethylsulfonylfluoride), Gpp(NH)p (5'-guanylylimidodiphosphate), leupeptin and pepstatin were obtained from Sigma, Chemical Company, St. Louis, M.O., U.S.A. Nuclear K_2 emulsion was purchased from Ilford, Amsterdam, The Netherlands. All other chemicals were of analytical grade.

Patients

Biopsies of human nasal mucosa from behind the inferior turbinate were obtained during sinus and septal surgery. Patients were classified into a non-allergic and an allergic group on the basis of the following parameters (Mygind, 1978; Passali, 1983); nasal symptoms, family history, X-rays of the sinuses, serum IgE, blood and nasal eosinophils, RAST and skin-tests. The non-allergic patients were further subdivided into control, chronic sinusitis and vasomotor rhinitis patients (table 9.1). The control group consisted of patients with a history of nasal trauma, with deviations of the septum and/or with cosmetic problems, and were characterized by low serum IgE values, few eosinophils in the blood or in the nose and negative RAST and skin-tests. The X-rays of the sinuses of chronic sinusitis patients demonstrated an inflammatory change but the other characteristics were similar to the control group. The pathophysiology of the vasomotor rhinitis group resembles the pathophysiology of allergic rhinitis but an allergic component can be excluded. All these patients showed nasal obstruction and the other characteristics of this group were similar to those of the control group. Allergic rhinitis was diagnosed on high IgE values, an increase in eosinophils in the blood and/or the nasal mucosa and a positive RAST and/or skin-test for house dust mites, pollen and/or dander.

Table 9.1: Characteristics of non-allergic (NA) and allergic rhinitis (A) patients

	n	Age	M/F	Medication	Sm	skin-test	IgE (U/ml)	Eo blood ($x10^6$ c/ml)	Eo nose (%)
Homogenates									
NA									
c	12	32±8	7/5	-	7	neg(12)	6-149(6)	45-265(5)	0-5(5)
s	3	39±21	3/0	Te(1)	1	neg(3)	14(1)	70/150(2)	0(1)
v	3	26±1	0/3	Vi(2)Be(1)	0	neg(3)	22-96(3)	110-180(3)	0(3)
A	11	29±11	8/3	Me(1)Pe(1)Ti(1)	2	HM(9)Po(7) Da(8)	142-1235(7)	34-1320(9)	0-60(9)
Autoradiography									
NA	2	59/67	2/0	Au(1)La(1)	0	neg(2)	ND	ND	ND
A	2	9/19	1/1	Lo(1)	0	HM(2)Po(1) Da(1)	440(1)	200/1900	15/2

A=Allergic rhinitis patients, Au=augmentine, Be=Beconase, c=control individuals, Da=human or animal dander, Eo=eosinophils, HM=house dust mite, La=Lanoxin, Lo=Lomudal, Me=methyrit, Pe=Penicillin, NA=non-allergic patients, ND=not determined, Po=Pollen, s=chronic sinusitis patients, Sm=smoker, Te=Tetracycline, Ti=Tinset, v=vasomotor rhinitis patients, Vi=Vibramycine, ( )=number of patients.

Radioligand receptor binding to homogenates of nasal mucosa.

Biopsies from the nasal mucosae were immediately washed in 0.9% NaCl after removal. The tissue was stored at -80°C for a maximum of 40 months (non-allergic 17±10 months; allergic 11±9 months). In rat nasal mucosa, no differences in binding parameters could be observed in fresh tissue in comparison with frozen mucosa. Similar binding parameters were obtained with human nasal mucosa stored for 40 months in comparison with 6 months. The binding procedure to human nasal mucosa was analogous to the procedure as described for rat nasal mucosa (chapter 2). The tissue was homogenized in icecold HEPES-PI buffer (20 mM HEPES, 120 mM NaCl, 10 mM $MgCl_2$, 1 mM PMSF, 1 mM EDTA, 0.01 mM leupeptin and 0.01 mM pepstatin) with an Ultraturrax for 2x10 sec. The homogenate was centrifuged at 1000xg for 5 min and the supernatant centrifuged at 100,000xg for 1 hour (4°C). The pellet was resuspended by Potter homogenization in HEPES-PI buffer. Protein determination was performed according to Bradford (1976) using bovine serum albumin as a standard.

Radioligand receptor binding studies were carried out in conical plastic centrifuge tubes, containing 40 µl ^{3}H-l-QNB, 40 µl HEPES buffer and 320 µl tissue homogenate (12.5 mg/ml). Non-specific binding was determined in the presence of 12 µM l-QNB. After the incubation at 37°C for 90 min the homogenates were centrifuged for 15 min at 18,000xg. Aliquots (250 µl) of the supernatants were mixed with 10 ml Aqua Luma (Lumac) for determining free concentrations of radioligand. The remaining supernatant was removed by suction. The tips of the centrifuge tubes were mixed with 10 ml Aqua Luma. The binding parameters were calculated by subjecting the data to a non-linear least squares curve fitting procedure using the Gauss Newton algorithm (Fletcher and Powell, 1963). Inhibition curves were analysed according to a one or two binding sites model.

Histology

Specimens of human nasal mucosae were washed in 0.9% NaCl and fixed in 2.5% glutardialdehyde in 0.1 M phosphate buffer (pH 7.0), dehydrated in ethanol and embedded in glycolmethacrylate (JB4 embedding kit, Polysciences Inc., Warrington, USA). Sections (2µm) were stained with 2.5% Toluidine Blue in 2.5% anhydrous sodiumdicarbonate.

In vitro labelling of muscarinic receptors in cryostat sections and autoradiography

Biopsies from the nasal mucosae were immediately washed in 0.9% NaCl after removal. The tissue was rapidly frozen using isopentane cooled in liquid nitrogen and stored for a maximum of 2 months for in vitro autoradiography.

Binding characteristics were examined before autoradiographic experiments were performed as described for rat nasal mucosa (van Megen et al., 1988). In summary, cryostat sections (10 μm) were thaw-mounted on glass slides coated with gelatin/chrome-alum. The sections were air dried and stored at -80°C. Before incubation the sections were dried again at room temperature and fixed at 4°C in 0.5% glutardialdehyde in Tyrode buffer for 30 min. Subsequently, the sections were rinsed in buffer at 4°C for 30 min and further processed for either radioreceptor assays or autoradiography. Several non-mounted parallel sections were collected for protein determination according to Lowry et al. (1951) with bovine serum albumin as a standard. Protein determination was performed after solubilization of the sections in 0.1 N NaOH at 100°C (boiling water-bath) for 15 min. Parallel sections were stained with methylgreen pyronin (1%) for histological verification of the tissue quality.

For the radioreceptor assay the tissue sections were overlayed with 150 μl ^{3}H-l-QNB in Tyrode buffer and incubated at 37°C for 90 min in a humid atmosphere. Non-specific binding was measured in the presence of 12 μM l-QNB under the same conditions. After the incubation, 75 μl of the incubation buffer was mixed with 10 ml Aqua Luma (Packard) to determine the concentration of radioligand. The remaining incubation buffer was removed by rinsing the sections in fresh buffer (3x4 min). The slides were broken, transferred to vials and the sections were solubilized overnight in 1 ml Soluene-350 (Packard) at 37°C. Instagel/0.5 N HCl (9:1) 18 ml, was added and the samples were counted in a liquid scintillation counter (1215 Rackbeta, LKB). The binding parameters were calculated as described for homogenates.

For the autoradiography, 10 μm sections of human nasal mucosae were incubated with 0.06 nM ^{3}H-l-QNB. Adjacent sections were incubated with 12

μM l-QNB in addition to ^{3}H-l-QNB to determine the non-specific binding. After incubation, the sections were rinsed in Tyrode buffer (room temperature) then briefly in distilled water to remove the buffer salts. The specimens were coated with 0.5% gelatin/chrome-alum and covered with nuclear K_2 emulsion. After an exposure time of 6 months (4°C) the autoradiograms were developed in amidol (18°C; 8 min), rinsed in distilled water, fixed in 30% sodiumthiosulphate (18°C; 8 min), thoroughly washed in distilled water (15 min) and stained with 1% methylgreen pyronin.

Statistical analysis

Agonist inhibition curves were fitted to a one or two binding sites model. Preference was given to a two binding sites model when a significant diminution in the residual sum of squares was reached (F-test). Differences in affinity values (negative logarithm of Kd) and receptor density (Bmax), which are known to be normally distributed (Fleming et al., 1972; Hancock et al., 1988), were compared using the student t-test. Significance was accepted at $p<0.05$.

9.4. Results

Muscarinic receptor characteristics in human nasal mucosa membranes

Specific ^{3}H-l-QNB binding to nasal mucosa membranes was saturable and of high affinity in non-allergic and allergic patients (fig. 9.1). The Scatchard plot of the specific binding (fig. 9.1) points to an homogeneous class of binding sites. Analysis of the data of non-allergic patients demonstrated a mean dissociation constant (Kd) of 47.2±4.4 pM and a receptor density (Bmax) of 2.65±0.3 pmol/g tissue or 616±52 fmol/mg protein (n=18)(table 9.2). In allergic patients, the Kd-value and the Bmax, expressed in pmol/g tissue or fmol/mg protein, were significantly lower than those determined in non-allergic patients.

The heterogenous non-allergic group was subdivided into control individuals (n=12), chronic sinusitis (n=3) and vasomotor rhinitis patients (n=3). The Kd- and Bmax-values were not significantly different between these subgroups. In allergic patients, the Kd-value and the Bmax,

expressed in pmol/g tissue or fmol/mg protein, were significantly decreased in comparison with those determined in control individuals. The Bmax, expressed in pmol/g tissue was significantly lower in allergic patients in comparison with chronic sinusitis patients. No other significant changes in Kd or Bmax could be observed in allergic patients in comparison with chronic sinusitis and with vasomotor rhinitis patients (table 9.2), probably due to the restricted number of these patients. Detailed analysis of the data revealed similar binding parameters from individuals on medication in comparison to those without in the non-allergic, control or allergic rhinitis group. Differences in binding parameters have neither been demonstrated between smokers in comparison with non-smokers in these groups.

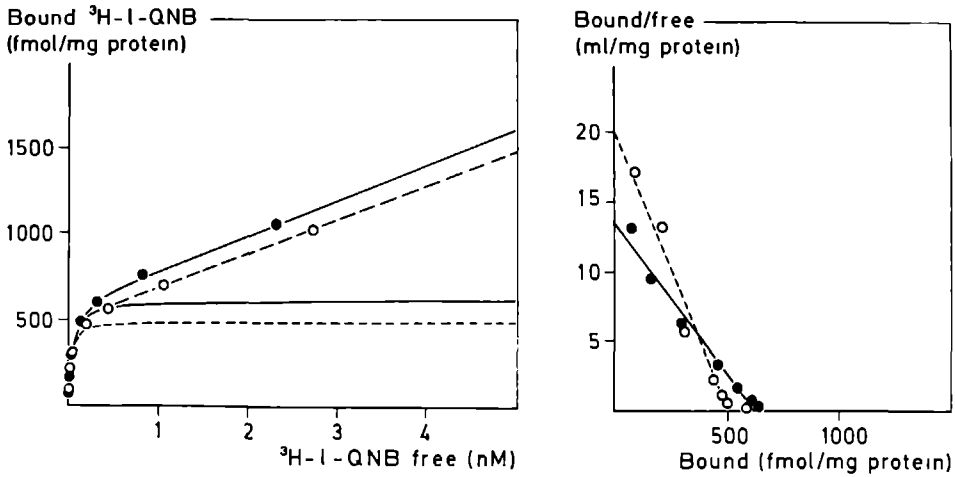


Fig. 9.1: Left: Total (●, O) and specific binding of ^{3}H-l-QNB to nasal mucosa membranes of non-allergic (——) and allergic (- -) patients. Non-specific binding was determined in the presence of 12 μM l-QNB. Right: Scatchard plot of the specific part of the binding. Data shown are the means of triplicates from representative experiments.

Table 9.2: Binding parameters (mean±SEM) of ^{3}H-1-QNB binding to muscarinic receptors in the nasal mucosa of non-allergic and allergic rhinitis patients.

	Kd (pM)	Bmax (t)	Bmax (p)	n
non-allergic	47.2±4.4*	2.65±0.30*	616±52*	18
control	49.2±5.5°	2.59±0.39°	661±68°	12
chronic sinusitis	39.3±9.4	3.25±0.58#	543±146	3
vasomotor rhinitis	47.0±15.1	2.27±0.87	449±51	3
allergic	35.0±5.5*°	1.56±0.29*°#	445±57*°	11

Bmax (t) in pmol/g tissue, Bmax (p) in fmol/mg protein, *o# $p<0.05$.

Inhibition studies of ^{3}H-1-QNB binding with the agonist methylfurtrethonium in the absence or presence of Gpp(NH)p were performed to investigate the agonist affinity states and the coupling of the muscarinic receptors to the G-proteins in non-allergic and allergic patients. The inhibition curve appeared to be biphasic in 2 non-allergic and 1 allergic patient and could significantly better be fitted according to a two binding sites model, indicating the presence of high and low affinity states (table 9.3). However, in 2 other non-allergic and 1 allergic patient the agonist inhibition curve displayed a monophasic character, which could not be significantly better fitted according to a two binding sites model (table 9.3). In the presence of 5.10^{-4} M Gpp(NH)p a shift of the monophasic inhibition curve was observed and a complete conversion of high into low affinity sites was observed in the biphasic curves (fig. 9.2). No changes in agonist inhibition or coupling to G-proteins could be observed in allergic patients (table 9.3).

Table 9.3: Inhibition of ^{3}H-l-QNB binding by the agonist methylfurtrethonium in the absence and presence of Gpp(NH)p in the nasal mucosa of non-allergic and allergic rhinitis patients.

	-Gpp(NH)p			+Gpp(NH)p
	Ki_1 (μM)	Ki_2 (μM)	%RH	Ki (μM)
non-allergic	0.2	10.9	17	24.3
	1.3	11.2	18	18.1
	-	12.2	-	41.0
	-	29.5	-	52.1
allergic	0.8	11.0	15	20.5
	-	26.2	-	131

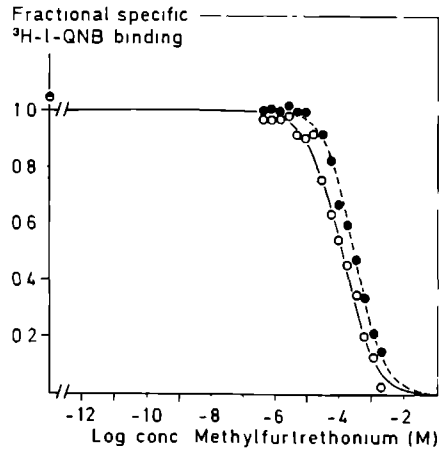


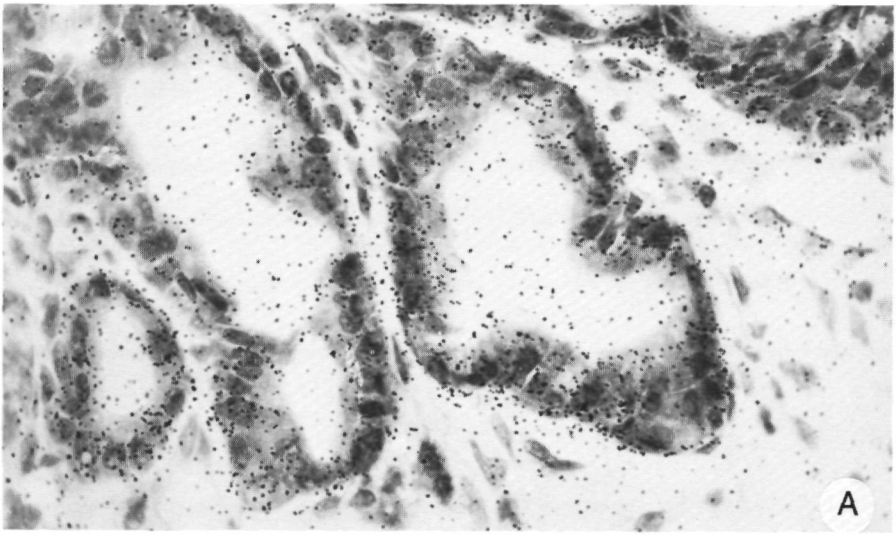
Fig. 9.2:
Inhibition of specific ^{3}H-l-QNB binding to nasal mucosa membranes of a non-allergic patient by the agonist methylfurtrethonium in absence (O) or presence (●) of 5.10^{-4} M Gpp(NH)p. The curve in the absence of Gpp(NH)p could significantly better fitted according to a two binding sites model, whereas the curve in presence of Gpp(NH)p could adequately be fitted according to a one binding site model.

Histology

Various tissue components were present in biopsies of human nasal mucosa; epithelium and the lamina propria with glands, blood vessels and inflammatory cells. Nervous tissue could not be observed. Biopsies of patients with allergic rhinitis demonstrated basophilic cells in the epithelium and an increased number of basophilic cells in the lamina propria in comparison with non-allergic patients. No other histological changes have been observed.

Localization of muscarinic receptors in human nasal mucosa.

Before the autoradiography, radioligand receptor binding experiments to cryostat sections of human nasal mucosa were performed. The saturation curves were used to determine the optimal free concentration radioligand for the in vitro autoradiography. Specific ^{3}H-l-QNB binding to muscarinic receptors was saturable and of high affinity in cryostat sections of 2 non-allergic individuals (Kd=340/400 pM; Bmax=45/40 fmol/mg protein) and 2 allergic patients (Kd=240/90 pM; Bmax=32/25 fmol/mg protein). After 6 months exposure the autoradiograms showed labelling of the glandular acini with ^{3}H-l-QNB (fig. 9.3A) in sections of non-allergic patients. Epithelium, blood vessels and connective tissue were devoid of silver grains. Autoradiographs made from sections incubated with an excess of unlabelled ligand in addition to ^{3}H-l-QNB showed only a very faint homogeneous labelling of the glandular tissue (fig. 9.3B). The autoradiographs showed similar specific labelling of the glandular acini in sections of the allergic patients.



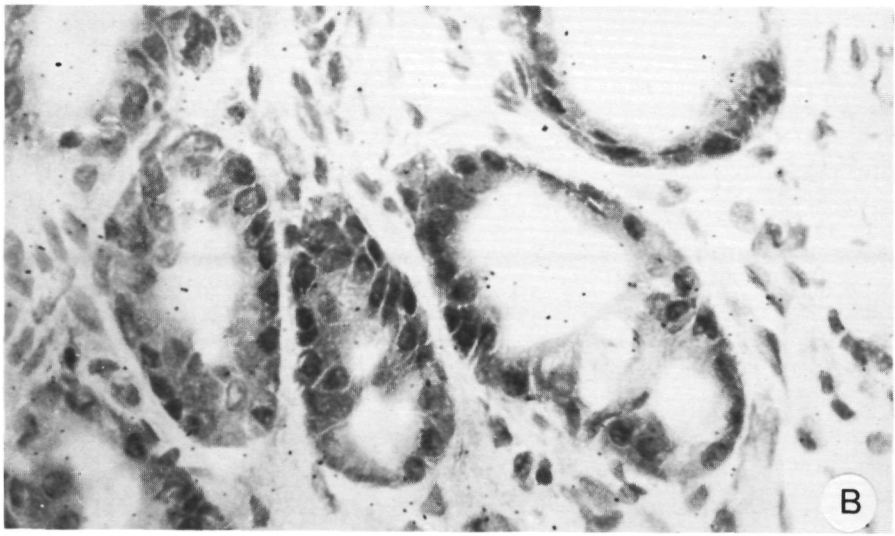


Fig. 9.3:
Autoradiograms of 0.5% glutardialdehyde-fixed cryostat sections (10 µm) of the nasal mucosa of a non-allergic patient after incubation with 0.8 nM ^{3}H-l-QNB (A) or with an excess of l-QNB in addition to the radioligand (B) followed by dipping in liquid nuclear emulsion. The autoradiograms show specific labelling of the glandular acini (x800). Staining with methylgreen pyronin (1%).

9.5. Discussion

Cholinergic nasal hyperresponsiveness has been observed in allergic patients (Borum et al., 1983; Druce et al., 1985; Corrado et al., 1986; Devillier et al., 1988) and may be due to an imbalance of the autonomic regulation (Mygind, 1982). In this study, the hypothesis of changes in muscarinic cholinergic characteristics and localization have been investigated. In the first instance, the biochemical characteristics and the autoradiographic localization of muscarinic receptors have been investigated in rat nasal mucosa, because of the limited supply of human nasal mucosa (Rodrigues de Miranda et al., 1985; Klaassen et al., 1986; van Megen et al., 1988).

The ^{3}H-l-QNB binding to muscarinic receptors in membranes of the nasal mucosa of non-allergic patients was saturable and of high affinity. The Kd-values were in agreement with those observed in rat nasal mucosa (Rodrigues de Miranda et al., 1985; Klaassen et al., 1986), human trachea smooth muscle (van Koppen et al., 1985) or human lung (Joad and Cassale, 1987). ^{3}H-l-QNB binding has been demonstrated in human nasal mucosa (Kd=3.9 nM, Ishibe et al., 1983; Kd=1.54 nM, Konno et al., 1987). The Kd-values reported were considerably higher, partly due to the use of the racemic ^{3}H-QNB instead of the levorotatory isomer as used in the present study.

The heterogeneous group of non-allergic patients was subdivided into control individuals, chronic sinusitis and vasomotor rhinitis patients. No significant differences in Kd-value or receptor density were demonstrated between these subgroups, probably due to the limited number of patients in the last 2 subgroups. The Kd-value and the receptor density were significantly decreased in allergic patients in comparison with non-allergic and control individuals. The increased sensitivity may reflect the cholinergic induced hypersecretion in nasal hyperreactivity. The decreased number of muscarinic receptors was somewhat surprising, since methacholine provocation resulted in a significant increased secretion in allergic patients. However, this decreased receptor number may reflect an adaptation of the effector cells to overstimulation or changes in density of inhibitory presynaptic

receptors. Inhibitory presynaptic muscarinic receptors have been demonstrated in lung (Fryer and Maclagan, 1984) and other tissues (Kilbinger, 1984). The small shifts in affinity and receptor density may reflect the cholinergic hyperreactivity in nasal allergy but are probably too small to explain the complex allergic reaction. A difference in sensitivity to methacholine of two glandular parts in the rat nasal mucosa was also accompanied by small shifts (factor 1.2) in receptor density and in affinity (Klaassen et al., 1987). No changes in the affinity but an increased density of muscarinic receptors have been reported by Japanese investigators (Isibe et al., 1983; Konno et al., 1987) in nasal mucosa of allergic patients in comparison with chronic sinusitis patients. The use of chronic sinusitis patients as controls may be responsible for the discrepancies between both studies, although no significant differences between control individuals and chronic sinusitis patients could be demonstrated in the present study. On the other hand, the discrepancy between both studies can possibly be explained by differences in the biopsy removal or medication. However, detailed analysis of the data revealed similar binding parameters from individuals with medication in comparison with individuals without medication in the non-allergic, control or allergic rhinitis group.

The agonist inhibition curves of ^{3}H-l-QNB binding to muscarinic receptors in homogenates of 2 out of 4 non-allergic and 1 out of 2 allergic patients demonstrated high affinity sites (17/18% and 15% respectively). The Ki-values of the high and low affinity sites were in agreement with those determined in rat nasal mucosa (chapter 2) or human tracheal smooth muscle (van Koppen et al., 1985). Generally, a heterogeneity of agonist binding has been a predominant feature of muscarinic receptors in a variety of tissues (Birdsall, 1984; McKinney and Richelson, 1984; Nathanson, 1987). However, the agonist inhibition curves of 3 patients demonstrated only low affinity binding sites. The absence of the high affinity sites is probably not due to proteolytic activity since protease inhibitors (PMSF, EDTA, leupeptin and pepstatin) were included. A small proportion (about 10%) of high affinity sites, admixed with low affinity sites, might be present but not detectable in such experiments. It has been proposed that the high affinity complex

consists of agonist, receptor and G-protein (Birdsall, 1984; Nathanson, 1987). In the presence of 5.10^{-4} M Gpp(NH)p a shift of the monophasic inhibition curve and a complete conversion of high into low affinity sites in biphasic curves was observed in non-allergic and allergic patients. This complete conversion has been demonstrated in rat nasal mucosa (chapter 2) and in other tissues (Birdsall, 1984; Nathanson, 1987). The increased sensitivity of allergic patients to cholinergic agonists might be reflected in the agonist binding. In the limited number of patients studied, no differences of agonist binding or coupling to the G-protein between allergic and non-allergic patients could be observed. In the lower airways, differences in agonist binding or coupling to G-proteins between chronic obstructive bronchitis patients and controls could neither be observed (Raaijmakers et al., 1984; van Koppen et al., 1989)

Binding of ^{3}H-l-QNB to human nasal mucosa cryostat sections was saturable and of high affinity. The saturation curves were used to determine the optimal free radioligand concentration for the autoradiography. The Kd-value of ^{3}H-l-QNB binding to the sections of human nasal mucosa is higher in comparison with the Kd-value of the ^{3}H-l-QNB binding to homogenates of human nasal mucosa. This phenomenon was also observed in rat nasal mucosa and it has been suggested that the affinity of the ligand for the receptor in more or less intact membranes of cryostat sections could be different from that in homogenized membranes (van Megen et al., 1988). The muscarinic receptor density in homogenates is higher than in sections, probably due to differences in protein contents; homogenates consist of a relatively pure membrane fraction, whereas sections contain all tissue constituents.

After an exposure time of 6 months, the autoradiographs of 10 μm sections of non-allergic patients showed specific ^{3}H-l-QNB labelling of the glandular acini. This observation provides evidence for the presence of muscarinic receptors in human nasal glands. The findings are in agreement with the in vitro autoradiographic results of rat nasal mucosa (van Megen et al., 1988) and with histochemical studies in the human nasal glands (Ishii and Toriyama, 1972; Cauna, 1982, Vecerina et al., 1983). The glandular acini produce (glyco)proteins (Thaete et al., 1981; chapter 7),

which are responsible for the viscosity and gel forming properties of the mucus (Clamp et al., 1978). From histochemical and autoradiographic experiments it may be concluded that the (glyco)protein production by the acini in human nasal mucosa is under parasympathetic control. Physiological studies reported also the cholinergic stimulation of nasal glycoprotein release in man (Patow et al., 1984). Histochemical studies demonstrated acetylcholine-esterase containing fibres in the sub-epithelial plexus (Ishii and Toriyama, 1972) and blood vessels (Cauna, 1982; Vecerina et al., 1983). The autoradiographs in this study did not show specific ^{3}H-l-QNB labelling of the epithelium or blood vessels.

No differences could be observed in the localization of muscarinic receptors in autoradiographs of allergic patients in comparison with those of controls. From radioligand and in vitro autoradiographic experiments it may be concluded that the number of muscarinic cholinergic receptors in the glandular acini are decreased in allergic patients in comparison with non-allergic and in comparison with control individuals. This changed receptor density may be a consequence of histological changes in the submucosal glands of allergic patients. However, no hyperplasia or hypertrophy of the nasal glands of the inferior turbinate could be observed in allergic patients in this or other studies (Mygind, 1974; Tos and Mogensen, 1977).

In summary, the increased sensitivity and the decreased number of muscarinic receptors may reflect the cholinergic induced hypersecretion in nasal allergy. No differences in agonist binding, coupling to G-proteins or localization of muscarinic receptors could be demonstrated in nasal allergy.

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ALTERATIONS OF ADRENOCEPTORS IN THE NASAL MUCOSA OF ALLERGIC PATIENTS IN COMPARISON WITH NON-ALLERGIC INDIVIDUALS.

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10.1 Abstract

Nasal hyperreactivity in nasal allergy may be due to changes of the characteristics in adrenergic receptors. Radioligand receptor binding studies with the antagonists ^{3}H-Prazosin (α_1), ^{3}H-Rauwolscine (α_2), ^{125}I-(-)-CYP (β) were performed in homogenates of nasal mucosa of allergic and non-allergic patients to investigate this hypothesis. The heterogeneous non-allergic group was subdivided into control, chronic sinusitis and vasomotor rhinitis patients. No significant differences in affinities or densities of α_1- and α_2-adrenoceptors could be demonstrated in allergic patients in comparison with non-allergic and control individuals.
The β-adrenoceptor density, was significantly reduced in allergic patients in comparison with control individuals. Neither changes in agonist binding or in the effect of Gpp(NH)p on the agonist binding to β-adrenoceptors could be observed in allergic patients. The subtype selective antagonist $LK_{203-030}$ demonstrated the presence of an homogeneous population of β_2-adrenoceptors in human nasal mucosa of both non-allergic and allergic patients. In vitro autoradiography demonstrated specific ^{125}I-(-)-CYP labelling of the epithelium in non-allergic and allergic patients. No specific labelling could be observed in the glands, blood vessels or connective tissue.
In conclusion, no changes in the characteristics of α_1- or α_2-

adrenoceptors in the nasal mucosa could be demonstrated in nasal allergy. However, a decreased number of β-adrenoceptors may reflect a β-adrenergic abnormality in nasal allergy.

10.2 Introduction

Nasal hyperreactivity in allergic rhinitis may originate from an imbalance of the autonomic nerve regulation in the nasal mucosa (Mygind, 1982). Systemic adrenergic abnormalities have been observed in allergic patients. Isoprenaline (β-agonist) infusion resulted in a significant decrease of pulse pressure and cAMP concentration in allergic patients in comparison with controls (Kaliner et al., 1982; Shelhamer et al., 1983). Intranasal administration of terbutaline (β_2-agonist) or phenylephrine (α_1-agonist) caused no differences in nasal airway resistance in allergic patients (Svensson et al., 1980; Brooks et al., 1988). Until now, the influence of β-adrenergic agonists on nasal secretion in allergic patients in comparison with controls has not been investigated. Beta-adrenergic hyporesponsiveness and alpha-adrenergic hyperresponsiveness in the lower airways have been explained in terms of changes in the characteristics of β- and α-adrenoceptors in patients with chronic airflow obstruction (Szentivanyi et al., 1979; Barnes et al., 1980; Raaijmakers et al., 1987; Meurs et al., 1987; Barnes et al., 1988). In contrast with the lower airways, only limited quantitative data (receptor densities) of α_1- and β-adrenoceptors in the human nasal mucosa are available (Ishibe et al., 1983; Konno et al., 1987). In this study radioligand receptor binding studies were performed with the antagonists ^{3}H-Prazosin, ^{3}H-Rauwolscine and ^{125}I-(-)-CYP to investigate α_1-, α_2- and β-adrenoceptors respectively in non-allergic and allergic patients in order to elucidate the supposed alteration in characteristics of neuroreceptors in nasal hyperreactivity. Furthermore, the heterogeneous non-allergic group has been subdivided into control individuals, chronic sinusitis and vasomotor rhinitis patients. The investigation of the β-adrenoceptors includes not only the receptor density and sensitivity but also the presence of β-adrenoceptor subtypes, agonist affinity states and the coupling of this receptor to the effector system by the G-

protein. In vitro autoradiography was performed to localize β-adrenoceptors in the human nasal mucosa of non-allergic and allergic patients.

10.3 Materials and methods

^{3}H-Prazosin (specific activity 18.8 Ci/mmol), ^{3}H-Rauwolscine (specific activity 73.5 Ci/mmol) and ^{125}I-(-)-CYP (^{125}I-Cyanopindolol)(specific activity 2200 Ci/mmol) were purchased from New England Nuclear, Doorn, The Netherlands. (±)-propranolol was a gift from ICI Ltd., Alderly Park, England; (±)-isoprenaline and $LK_{203-030}$ were gifts from Boehringer Ingelheim, FRB and Sandoz, Basel Switzerland respectively. Phentolamine was a gift from Ciba-Geigy, N.J. Gpp(NH)p (5'-guanylylimidodiphosphate) and PMSF (phenylmethylsulfonylfluoride) were obtained from Sigma Chemical Co., St. Louis, M.O., U.S.A. Nuclear K2 emulsion was purchased from Ilford, Amsterdam, The Netherlands. All other chemicals were of analytical grade.

Patients

Biopsies of human nasal mucosa from behind the inferior turbinate were obtained during sinus and septal surgery. Patients were classified into a non-allergic and allergic group on the basis of the following parameters (Mygind, 1978; Passali, 1983); nasal symptoms, family history, X-rays of the sinuses, serum IgE, blood and/or nose eosinophilia, RAST and skin-tests. The non-allergic patients were further subdivided into control, chronic sinusitis and vasomotor rhinitis patients (table 10.1). The control group consisted of patients with a history of nasal trauma, deviations of the septum and cosmetic problems, and were characterized by low serum IgE values, few eosinophils in the blood or in the nose and negative RAST and skin-tests. The X-rays of the sinuses of chronic sinusitis patients demonstrated an inflammatory change but the other characteristics were similar to the control group. The pathophysiology of the vasomotor rhinitis group resembled the pathophysiology of allergic rhinitis but an allergic component could be excluded. All these patients showed nasal obstruction and the other characteristics of this group were

Table 10.1: Characteristics of non-allergic (NA) and allergic rhinitis (A) patients

	n	Age	M/F	Medication	Sm	skin-test	IgE (U/ml)	Eo blood ($x10^6$ c/ml)	Eo nose (%)
ß-adrenoceptors									
NA									
c	13	31±9	5/8	Bi(1)Be(1)L(1)	4	neg(13)	6-149(8)	45-165(8)	0-5(8)
s	3	40±25	2/1	Be(1)	0	neg(3)	28(1)	50/226(2)	0(1)
v	2	26/28	0/2	Vi, Tr(1)Be(1)	0	neg(2)	110(2)	110/180(2)	0(2)
A	14	23±4	9/5	Ve(2)Be(3)Ti(2)	2	HM(10) Po(10)Da(10)	155-4650(12)	60-685(11)	0-9(11)
α_1-adrenoceptors									
NA									
c	5	29±12	2/3	Be, Ot(1)	1	neg(5)	6-49(4)	90-165(4)	0-3(4)
s	1	17	1/0	no	0	neg(1)	ND	ND	ND
v	1	26	0/1	Vi, Tr(1)	0	neg(1)	22(1)	110(1)	0(1)
A	6	22±6	4/2	Be(1)Ro(1)Ti(1)	0	HM(5) Po(4)Da(4)	226-1110(3)	100-685(3)	0-5(3)
α_2-adrenoceptors									
NA									
c	8	32±7	4/4	Be, Ot(1)	3	neg(8)	10-44(4)	80-120(3)	0-3(3)
s	3	33±15	2/1	Te(1)	2	neg(3)	51(1)	70/80(2)	0/70(2)
v	3	30±6	0/3	Vi,Tr(1)Be(1)L(1)	0	neg(3)	22-96(3)	110-273(3)	0-25(3)
A	9	26±9	6/3	Me(1)Pe(1)Ti(1)	1	HM(7) Po(6)Da(5)	226-1235(5)	286-1320(7)	0-60(7)

A=allergic rhinitis, Au=augmentine, Be=Beconase, Bi=Bisolvon, c=control, Da=human or animal dander, Eo=eosinophilia, HM=house dust mite, La=Lanoxin, L=Lomudal, Me=methyrit, NA=non-allergic, ND=Not determined, Ot=Otrivin, Pe=Penicillin, Po=Pollen, Ro=Ronnisol, s=chronic sinusitis, Sm=smoker, Te=Tetracycline, Ti=Tinset, Tr=Triludan, v=vasomotor rhinitis, Ve=Ventolin, Vi=Vibramycine, ( )=number of patients.

similar to those of the control group. Allergic rhinitis was diagnosed on high IgE values, an increase in eosinophils in the blood and/or the nasal mucosa and a positive RAST and/or skin-test for house dust mites, pollen and/or dander. None of the allergic patients demonstrated an acute clinical manifestation of the allergic rhinitis.

Radioligand receptor binding to homogenates of nasal mucosa

Biopsies from the nasal mucosae were immediately washed in 0.9% NaCl after removal. The tissue was stored at -80^{o}C for a maximun of 43 months (non-allergic 18±12 months, allergic 17±7 months). In rat nasal mucosa, no differences in binding parameters could be observed in fresh tissue in comparison with frozen mucosa. Similar binding parameters were obtained with human nasal mucosa stored for 36 months in comparison with 7 months. The binding procedures to human nasal mucosa were analogous to the procedures as described for rat nasal mucosa (van Megen et al., 1989; chapter 4 and 6). In summary, the tissue was homogenized in ice cold buffer with an Ultraturrax for 2x10 sec. The homogenate was centrifuged at 1000xg for 5 min, the supernatant centrifuged at 100,000xg for 1 hour (4^{o}C) and the pellet was resuspended by Potter homogenization. Protein determination was performed according to Bradford (1976) using bovine serum albumin as a standard.

Radioligand receptor binding studies were carried out in glass tubes containing 40 µl radioligand, 40 µl buffer and 320 µl tissue homogenate. The different incubation conditions for the various receptors are listed in table 10.2. Instead of the buffer, unlabelled ligand was added for the determination of the non-specific binding. Incubations were terminated by rapid dilution with 2x2 ml ice cold buffer and filtration through Whatman GF/C filters under vacuum, followed by 3x5 ml rinses of the filter with buffer. Radioactivity collected on each filter was measured in a gammacounter (NE1612, NEN) at 78% counting efficiency in case of the ^{125}I-ligand, whereas ^{3}H-radioactivity was counted in a liquid scintillation counter. The binding parameters were calculated by subjecting the data to a non-linear least square curve fitting procedure using the Gauss-Newton algorithm (Fletcher and Powell, 1963). Inhibition curves were analysed according to a one or two binding sites model.

Table 10.2: Incubation conditions for receptor assays of α- and β-adrenoceptors (R= drenoceptor).

Ligand	R	Buffer	Incubation	Non-specific binding
^{3}H-Prazosin	α_1	Tris/HCl	40 min, 25°C	12 μM phentolamine
^{3}H-Rauwolscine	α_2	Tris/EDTA	20 min, 25°C	12 μM phentolamine
^{125}I-(-)-CYP	β	Tyrode+0.01% BSA	90 min, 37°C	2 μM (±)propranolol

Histology

Specimens of human nasal mucosae were washed in 0.9% NaCl and fixed in 2.5% glutardialdehyde in 0.1 M phosphate buffer (pH 7.0), dehydrated in ethanol and embedded in glycolmethacrylate (JB4 embedding kit, Polysciences Inc., Warrington, USA). Sections (2μm) were stained with 2.5% Toluidine Blue in 2.5% anhydrous sodiumdicarbonate.

In vitro labelling and autoradiography of β-adrenoceptors in cryostat sections.

Biopsies from the nasal mucosae were immediately washed in 0.9% NaCl after removal. The tissue was rapidly frozen using isopentane cooled in liquid nitrogen and stored for a maximum of 2 months before in vitro autoradiography.

Binding characteristics were examined before autoradiographic experiments were performed as described for rat nasal mucosa (van Megen et al., 1988). In summary, cryostat sections (10 μm) were thaw-mounted on glass slides coated with gelatin/chrome-alum. The sections were air dried and stored at -80°C. Before incubation the sections were dried again at room temperature and fixed at 4°C in 0.5% glutardialdehyde in Tyrode buffer for 30 min. Subsequently, the sections were rinsed in buffer at 4°C for 30 min and further processed for either radioreceptor assays or autoradiography. Several non-mounted parallel sections were collected for protein determination according to Lowry et al. (1951) with bovine serum albumin as a standard. Protein determination was performed after solubilization of the sections in 0.1 N NaOH at 100°C (boiling water-bath) for 15 min. Parallel sections were stained with methylgreen

pyronin (1%) for histological verification of tissue quality.
For the radioreceptor assay, the tissue sections were dried and overlayed with 150 μl ^{125}I-(-)-CYP in Tyrode buffer (with 0.01% BSA) at various concentrations radioligand. Subsequently, the sections were incubated at 37^{0}C for 2 hrs in a humid atmosphere. For the measurements of the non-specific binding, parallel incubations were performed in the presence of 2 μM (±)-propanolol at the same conditions. After the incubation, 75 μl of the incubation buffer was counted to determine the free concentration radioligand, the remaining incubation buffer was removed by rinsing the sections in fresh buffer (r.t.) during 4x15 min. The radioactivity in the sections was counted by gamma counting (NE1612 gammacounter, NEN). The binding parameters were calculated as described above.
For the autoradiography, sections were incubated with 0.02 nM ^{125}I-(-)-CYP according to the method described above. After washing the slides in buffer, they were rinsed in distilled water to remove salts. The specimens were coated with 0.5% gelatin/chrome alum, air dried and dipped in liquid emulsion (Nuclear K_2 emulsion, Ilford) diluted with distilled water (1:1). After 2 months exposure (4^{o}C) the autoradiographs were developed in amidol (18^{0}C, 8 min), rinsed in distilled water, fixed in sodium thiosulphate (30%) at 18^{0}C for 1-3 min, thoroughly washed in distilled water (15 min) and stained with 1% methylgreen pyronin.

Statistical analysis

Agonist inhibition curves were fitted to a one or two binding sites model. Preference was given to a two binding sites model when a significant diminution in the residual sum of squares was reached (F-test). Differences in affinity values (negative logarithm of Kd, K_H and K_L), which are known to be normally distributed (De Lean et al., 1982; Hancock et al., 1988) were compared using the student t-test. Significance was accepted at $p<0.05$.

10.4. Results

Histology

Various tissue components were present in biopsies of human nasal mucosa; epithelium and the lamina propria with glands, blood vessels and inflammatory cells. Nervous tissue could not be observed in the specimens. Biopsies of patients with allergic rhinitis demonstrated basophilic cells in the epithelium and an increased number of basophilic cells in the lamina propria in comparison with non-allergic patients. No other histological changes have been observed.

Alpha-adrenoceptors in nasal mucosa membranes.

Specific binding of ^{3}H-Prazosin and ^{3}H-Rauwolscine to α_1-adrenoceptors and α_2-adrenoceptors respectively was saturable and of high affinity in non-allergic as well as allergic patients. Scatchard plots point to homogeneous classes of binding sites. The binding parameters, equilibrium dissociation constant (Kd) and the receptor density (Bmax) are listed in table 10.3. No significant differences in binding parameters could be observed in allergic patients in comparison with non-allergic individuals. The heterogeneous non-allergic group was subdivided into control individuals, chronic sinusitis and vasomotor rhinitis patients. The binding parameters of ^{3}H-Prazosin and ^{3}H-Rauwolscine binding were not significantly different between these subgroups or between allergic patients and the subgroups.

Beta-adrenoceptors in nasal mucosa membranes

Specific ^{125}I-(-)-CYP binding to β-adrenoceptors was saturable and of high affinity in nasal mucosa membranes of non-allergic and allergic patients (fig. 10.1). The Scatchard plot points to an homogeneous class of binding sites. Similar binding parameters were obtained in allergic patients and non-allergic individuals. The non-allergic group was divided in subgroups as described above. The β-adrenoceptor density, expressed in fmol/mg protein, was significantly decreased in allergic patients in comparison with control individuals (table 10.3).

Table 10.3: Binding parameters (mean±SEM) of ^{3}H-Prazosin, ^{3}H-Rauwolscine and ^{125}I-(-)-CYP to α_1-, α_2- and β-adrenoceptors in the nasal mucosae of non-allergic and allergic rhinitis patients.

α_1-adrenoceptors				
	Kd (nM)	Bmax (t)	Bmax (p)	n
non-allergic	0.4±0.1	0.77±0.14	177±33	5
control	0.4±0.2	0.90±0.17	195±40	3
chronic sinusitis	0.5	0.35	80	1
vasomotor rhinitis	0.2	0.83	218	1
allergic	0.3±0.1	0.80±0.11	244±50	4
α_2-adrenoceptors				
	Kd (nM)	Bmax (t)	Bmax (p)	n
non-allergic	2.7±0.5	5.53±1.04	1180±127	14
control	2.7±0.6	4.61±0.87	1070±119	8
chronic sinusitis	3.7±1.4	9.65±3.80	1630±430	3
vasomotor rhinitis	1.6±0.2	3.86±0.59	1020±198	3
allergic	2.3±0.4	5.21±0.59	1230±96	9
β-adrenoceptors				
	Kd (pM)	Bmax (t)	Bmax (p)	n
non-allergic	2.7±0.2	0.50±0.06	87±11	18
control	2.8±0.3	0.48±0.06	92±10*	13
chronic sinusitis	2.9±0.2	0.67±0.20	100±46	3
vasomotor rhinitis	2.7/1.6	0.32/0.45	42/30	2
allergic	3.1±0.4	0.41±0.05	63±6*	14

Bmax(t) in pmol/g tissue, Bmax(p) in fmol/mg protein *$p<0.05$

No significant differences in binding parameters could be demonstrated in allergic patients in comparison with chronic sinusitis and vasomotor rhinitis patients (table 10.3). Further analysis of the data revealed similar binding parameters in individuals with medication and in individuals without medication of the non-allergic, control or allergic rhinitis group nor could differences in binding parameters be demonstrated between smokers in comparison with non-smokers in these groups.

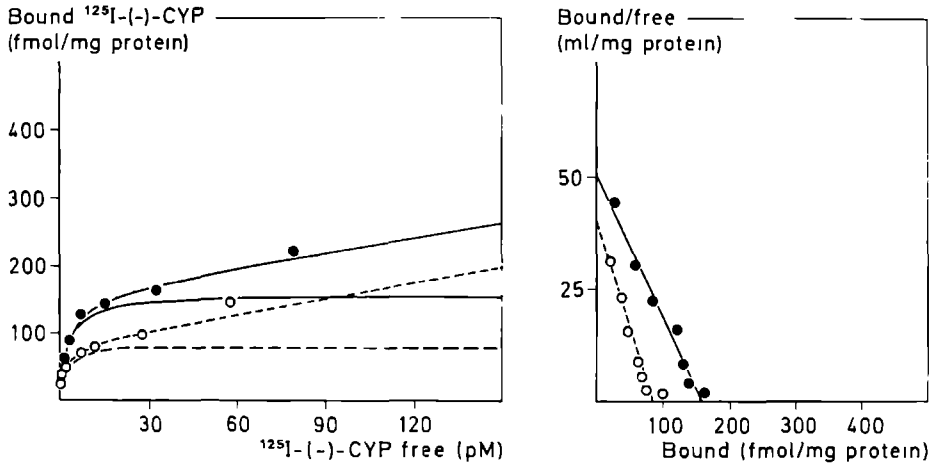


Fig. 10.1:
Left: Total (●, O) and specific ^{125}I-(-)-CYP binding to β-adrenoceptors in human nasal mucosa membranes of non-allergic (——) and allergic (- -) patients. Non-specific binding was determined in the presence of 2 μM (±)-propranolol. Right: Scatchard plot of the specific part of the binding to nasal mucosa membranes. Data shown are the means of triplicates from representative experiments.

The subtype selective β_1-adrenergic antagonist $IK_{203-030}$ was used to define the relative proportions of β_1- and β_2-adrenoceptors in human nasal mucosa. The inhibition curve was monophasic and could adequately be fitted according to a one binding site model in control and allergic patients (fig. 10.2). The low Ki-values (8.9±2.8 μM and 7.4±3.3 μM

respectively), indicated the presence of an homogeneous population of β_2-adrenoceptors in control individuals and allergic patients.

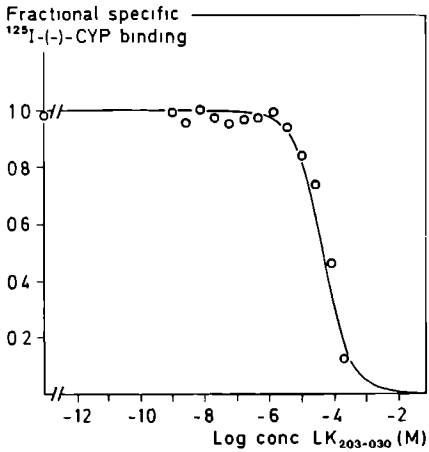
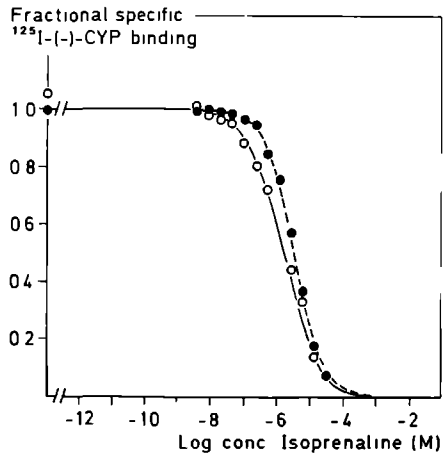


Fig. 10.2:
Inhibition of specific ^{125}I-(-)-CYP binding by the antagonist $LK_{203-030}$. Homogenates of nasal mucosa of non-allergic patients were incubated at 37°C for 120 min at 16 pM ^{125}I-(-)-CYP in the presence or absence of inhibitor. The inhibition curve could adequately be fitted by a one binding site model. Data shown are the means of triplicates from a representative experiment.

Fig. 10.3:
Inhibition of ^{125}I-(-)-CYP binding by the agonist isoprenaline in absence (O) or presence of 5.10^{-4} M Gpp(NH)p (●). The curve in the absence of Gpp(NH)p could significantly better fitted according to a two binding sites model, whereas the the curve in the presence of Gpp(NH)p could adequately be fitted according to a one binding site model.



Inhibition studies of ^{125}I-(-)-CYP with the agonist isoprenaline in the absence or presence of Gpp(NH)p were performed to investigate the agonist affinity states and the coupling of the β-adrenoceptors to the G-proteins in human nasal mucosa (table 10.4). The inhibition curve appeared to be biphasic in 4 out of 5 control individuals and 3 out of 4 allergic patients and could be significantly better fitted according to a two binding sites model, indicating the presence of 15±6% and 21±10% high affinity sites respectively. Monophasic inhibition curves were demonstrated in 1 control and 1 allergic patient. In the presence of 5.10^{-4} M Gpp(NH)p a shift of the monophasic and a steepening of the biphasic curves was observed (table 10.4). In two biphasic curves (1 control, 1 allergic patient) Gpp(NH)p caused a complete conversion of the high into the low affinity sites (fig. 10.3). No remarkable changes in agonist inhibition or coupling to G-proteins could be demonstrated in allergic patients.

Table 10.4: Inhibition of ^{125}I-(-)-CYP binding by the agonist isoprenaline in absence or presence of Gpp(NH)p in control (Co) and allergic rhinitis patients (Al).

	-Gpp(NH)p				+Gpp(NH)p			
	Ki (μM)	Ki (μM)	RH(%)	n	Ki (μM)	Ki (μM)	RH*(%)	n
Co	0.66	-	-	1	1.0	-	-	1
	0.016±0.024	0.48±0.23	15±6	4	0.16±0.11	1.93±0.81	42±28	3
					-	0.36	-	1
Al	0.19/0.17	-	-	2	0.22/0.44	-	-	2
	0.027±0.003	0.79±0.29	21±10	3	0.68±0.47	1.23/1.74	65/46	2
					-	0.60	-	1

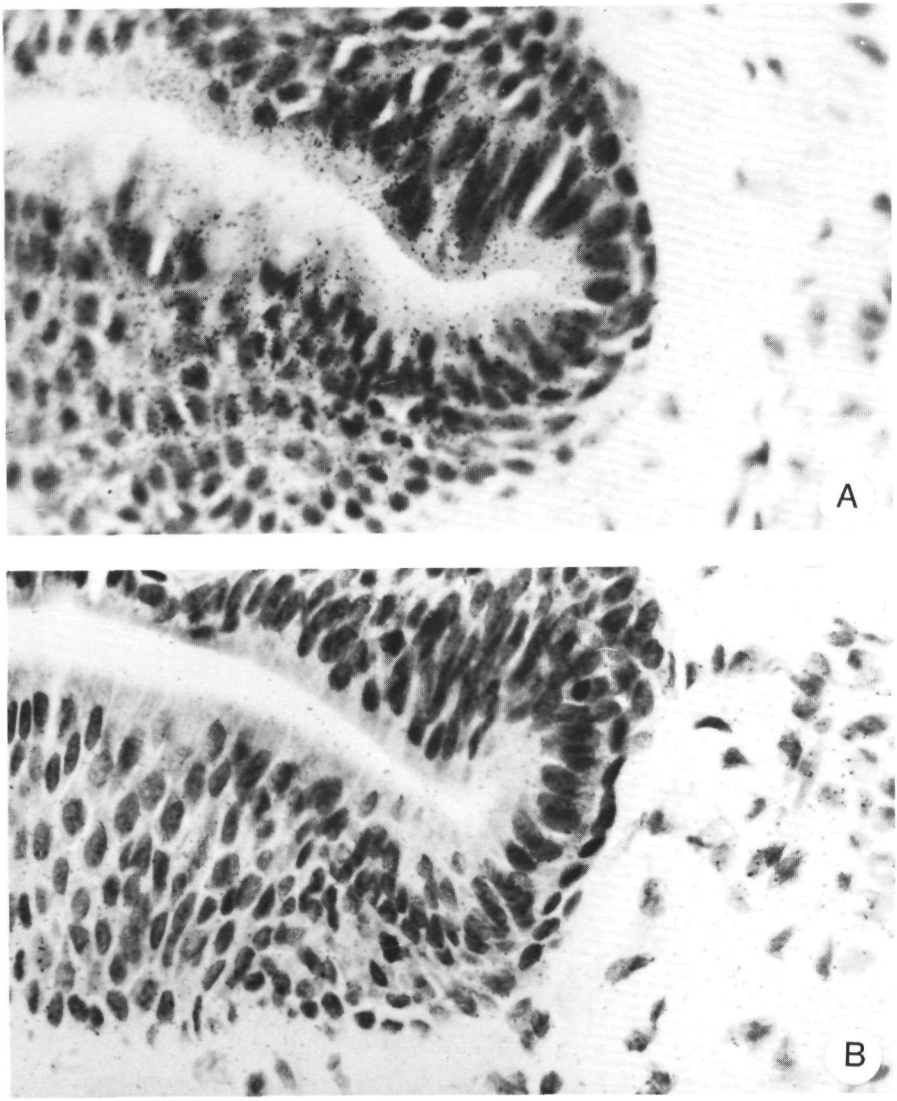


Fig. 10.4:
Autoradiograms of 0.5% glutardialdehyde fixed cryostat sections (10 µm) of the nasal mucosa of a non-allergic patient after incubation with 16 pM ^{125}I-(-)-CYP (A) or with 2 µM (±)-propranolol in addition to the radioligand (B) followed by dipping in the nuclear emulsion. The autoradiograms show specific labelling of the nasal epithelium (x800). Staining with methylgreen pyronin (1%).

Localization of β-adrenoceptors in human nasal mucosa

Before the autoradiography, radioligand receptor binding experiments to cryostat sections of human nasal mucosa were performed. The saturation curves were used to determine the optimal free concentration radioligand for the in vitro autoradiography. Specific ^{125}I-(-)-CYP binding to β-adrenoceptors was saturable and of high affinity in cryostat sections of non-allergic (Kd=4.3±2.4 pM; Bmax=3.7±2.8 fmol/mg protein; n=4) and allergic patients (Kd=6.3/5.8 pM; Bmax=6.3/3.5 fmol/mg protein; n=2). After 2 months exposure the autoradiographs showed ^{125}I-(-)-CYP labelling of the nasal epithelium of non-allergic as well as allergic patients (fig. 10.4). Autoradiographs made from sections incubated in the presence of 2 μM (±)-propranolol in addition to the radioligand showed only a faint homogeneous labelling of the nasal mucosa. Nasal glands, blood vessels, and connective tissue were devoid of silver grains.

10.5. Discussion

Nasal hyperreactivity in allergic patients (Kaliner et al., 1982; Shelhamer et al., 1983) may be due to an imbalance of the autonomic regulation (Mygind, 1982). In this study, the hypothesis of changes in adrenergic, α_1-, α_2- and β-adrenergic characteristics has been investigated. Changes of agonist binding, coupling to the G-protein and localization were only investigated for the β-adrenoceptors due to the unfavourable ratio specific/non-specific binding in the α-adrenergic assays. In view of the limited amount of human tissue, the rat nasal mucosa has been used as a model to investigate the biochemical characteristics of adrenoceptors and the localization of β-adrenoceptors (van Megen et al., 1988; chapters 4, 5 and 6).

^{3}H-Prazosin and ^{3}H-Rauwolscine binding to α_1- and α_2-adrenoceptors in membranes of the nasal mucosae of non-allergic patients was saturable and of high affinity. The Kd-values were in agreement with those observed in rat nasal mucosa (van Megen et al., 1989; chapter 6) or other human tissues (Yamada et al., 1987; Muller and Noack, 1988). The heterogeneous non-allergic group was subdivided into control individuals, chronic sinusitis and vasomotor rhinitis patients. Similar binding parameters of

the ^{3}H-Prazosin and ^{3}H-Rauwolscine binding were obtained in these subgroups. As high tissue concentrations (25 mg/ml) were required to observe specific ^{3}H-Prazosin binding only a limited number of patients could be investigated. No significant differences in affinities or densities of α_1- and α_2-adrenoceptors could be demonstrated in allergic patients in comparison with non-allergic and control individuals. As far as the α_1-adrenoceptors, these findings are in agreement with similar reactivity to α_1-agonists in allergic and non-allergic patients (Brooks et al., 1988). In the lower airways, α-adrenergic hyperresponsiveness has been explained by an increased density of α_1-adrenoceptors (Barnes et al., 1980). ^{3}H-Prazosin, but not ^{3}H-Rauwolscine binding, has been demonstrated in human nasal mucosa (Kd=0.31 nM, Ishibe et al., 1983; Kd=1.10 nM, Konno et al., 1987). These Japanese studies reported a decreased number of α_1-adrenoceptors in the nasal mucosa of allergic patients in comparison with chronic sinusitis patients. The discrepancy between both studies may be due to the use of chronic sinusitis patients as a control group or to differences in biopsy removal and/or in medication. In regard to the unchanged affinities and densities of α-adrenoceptors demonstrated in this study, alpha-agonists do not seem to be the appropriate anti-allergic drugs. Bende and Laurin (1986) also suggested that α-agonists are adequate as decongestants but not as specific anti-allergic drugs.

The Kd-values of the ^{125}I-(-)-CYP binding to β-adrenoceptors in membranes of the nasal mucosae of non-allergic and allergic patients were in agreement with those observed in rat nasal mucosa (chapter 4) and other human tissues (van Koppen et al., 1989). No significant differences of the binding parameters could be demonstrated in allergic patients in comparison with non-allergic individuals. However, if the non-allergic group was further subdivided, a significant decrease of the β-adrenoceptor density, expressed per mg protein, could be demonstrated in allergic patients in comparison with control individuals. These findings emphasize the importance of an accurate diagnosis of the patients. The expression of the receptor density per mg protein is probably more accurate than the expression per g tissue. The decreased number of β-adrenoceptors may reflect a β-adrenergic abnormality in nasal

allergy (Kaliner et al., 1982; Shelhamer et al., 1983). However, intranasal administration of terbutaline caused no changes in the nasal airway resistance in allergic patients in comparison with non-allergic patients (Svensson et al., 1980). Until now, the influence of β-adrenergic agonists on the nasal secretion in allergic patients in comparison with non-allergic patients has not been investigated. In the lower airways an infusion of terbutaline resulted in an increased mucociliary transport in asthmatic patients . The small shift in receptor density may reflect a possible β-adrenergic abnormality in nasal allergy but is probably too small to explain the complex allergic reaction. A decreased β-adrenoceptor density has been reported in allergic patients in comparison with chronic sinusitis patients with the antagonist ^{3}H-Dihydroalprenolol (Ishibe et al., 1983; Konno et al., 1987). In the present study, no significant difference in receptor density could be observed in allergic patients in comparison with chronic sinusitis patients, possibly due to the limited number of patients investigated. The discrepancy between both studies may also be explained by differences in biopsy removal and/or medication. However, further analysis of the data revealed similar binding parameters in individuals with medication and those without in the non-allergic, control or allergic rhinitis group.

The agonist inhibition curves of ^{125}I-(-)-CYP binding to β-adrenoceptors in nasal mucosa membranes of 4 out of 5 controls and 3 out of 4 allergic patients demonstrated 15±6% and 21±10% high affinity sites respectively. The Ki-values of the high and low affinity sites were in agreement with those observed in rat nasal mucosa (chapter 4) and human smooth muscle (van Koppen et al, 1989). Generally, a heterogeneity of agonist binding has been a predominant feature of β-adrenoceptors in a variety of tissues (Stiles et al., 1984; Levitzki, 1986). However, the agonist binding of 1 control and 1 allergic patient demonstrated only low affinity sites. A small proportion (less than 10%) of high affinity sites, admixed with low affinity sites, might be undetectable in such experiments. It has been accepted that the high affinity complex consists of agonist, receptor and G-protein. Guanine nucleotides appeared to convert high affinity states into low affinity states of the receptor (Stiles et al., 1984; Cheung et

al., 1989). In the presence of 5.10^{-4} M Gpp(NH)p a shift of the monophasic and a steepening of the biphasic agonist curves has been demonstrated. A complete conversion of the high into the low affinity sites has been observed in 2 patients. Since the protease inhibitor PMSF had no effect on the inhibition curve, proteolytic activity is probably not responsible for the incomplete conversion. Complete conversion has neither been demonstrated in rat nasal mucosa (chapter 4), bovine trachea smooth muscle (van Koppen et al., 1987) and rat heart (Christ et al., 1988). A β-adrenergic abnormality of allergic patients may be reflected in the agonist binding. However, no differences in agonist binding or in effect of Gpp(NH)p on the agonist binding between allergic and controls could be observed. The number of agonist inhibition curves must be extended for definite conclusions. In the lower airways, differences in agonist binding could neither be observed in patients with chronic airflow obstruction in comparison with controls (van Koppen et al., 1989). However, allergen challenge of patients with asthma resulted in a reduced β-adrenergic agonist binding in lymphocytes (Meurs et al., 1987). The antagonist and agonist binding to in vitro antigen challenged nasal mucosa of allergic patients give probably more information on an autonomic disbalance in the more acute manifestation of allergic rhinitis.

The Kd-values of the ^{125}I-(-)-CYP binding to cryostat sections of human nasal mucosa are similar to those observed in homogenates. The β-adrenoceptor density in homogenates is higher than in sections probably due to differences in protein contents; homogenates consist of a relatively pure membrane fraction, whereas sections contain all tissue constituents. The ^{125}I-(-)-CYP saturation curve was used for the determination of the optimal free concentration radioligand in autoradiography. After 2 months exposure, the autoradiographs of both non-allergic and allergic patients showed specific ^{125}I-(-)-CYP labelling of the epithelium. The presence of adrenoceptors in the epithelium of human and rat nasal mucosa has been demonstrated before by histochemical and autoradiographic experiments (Vecerina et al., 1983; chapter 4). Beta-adrenoceptors in airway epithelium may play a role in the mucociliary transport, active ion transport or the production of

epithelium derived factors (Davis et al., 1979; Carstairs et al., 1985; Welsh, 1987). No specific ^{125}I-(-)-CYP labelling could be observed in the nasal blood vessels, which is in agreement with other studies (Hall and Jackson, 1968). However, a small population of β-adrenoceptors may be present (Malm, 1977).

Inhibition studies with the subtype selective antagonist $LK_{203-030}$ in homogenates and in vitro autoradiography indicated that the ^{125}I-(-)-CYP labelling in the autoradiographs occurs to a homogeneous β_2-adrenoceptor population in epithelium of the human nasal mucosa. The combination of radioligand receptor binding and in vitro autoradiography suggests a decreased β-adrenoceptor density in the epithelium of allergic patients in comparison with controls. This finding is in agreement with quantitative autoradiographic results of the lower airways in chronic airflow obstruction (Raaijmakers et al., 1987).

Histological examination of the biopsies demonstrated mast cells in the epithelium of allergic patients, whereas no mast cells could be observed in non-allergic patients. Clinical pharmacological studies refer to the anti-allergic effect of β-agonists by inhibiting histamine release from mast cells (Kaliner and Austen, 1975). No specific ^{125}I-(-)-CYP labelling of mast cells could be observed in non-allergic and allergic patients in this study.

In summary, no changes in affinities or densities of α_1- or α_2-adrenoceptors in the nasal mucosa of allergic patients in comparison with control individuals could be demonstrated. The decreased number of β-adrenoceptors may reflect a β-adrenergic abnormality in allergic patients. No differences in agonist binding, coupling to G-proteins or localization of β-adrenoceptors could be demonstrated in nasal allergy.

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Nasal hyperreactivity in allergic rhinitis may originate from an imbalance of the autonomic nerve regulation by changes in characteristics of neuroreceptors in the nasal mucosa. In this study, radioligand receptor binding and in vitro autoradiographic assays were developed to determine receptor densities, sensitivities, subclasses, receptor-effector coupling and/or localization of the neuroreceptors in the nasal mucosa of non-allergic and allergic patients. Initially, the rat was used as an experimental model so that the restricted amount of human tissue was not a limiting factor.

Chapter 1 is a brief review of the anatomy, physiology and autonomic innervation of the nasal mucosa and also describes pharmacological and biochemical aspects of various neuroreceptors. Attention has also been paid to classification and mechanisms of nasal hyperreactivity.

In **chapter 2 and 3** the pharmacological characterization and the localization of muscarinic acetylcholine receptors in rat nasal mucosa is described. The muscarinic receptors were classified as M_3 receptors on the basis of the selective antagonists AF-DX-116 and HHSiD. High and low affinity agonist binding sites could be demonstrated after the inclusion of protease inhibitors during the assay. In vitro autoradiography demonstrated the presence of muscarinic receptors in the glandular acini and to a lesser extent in the excretory ducts.

Chapter 4 describes the pharmacological characterization and the localization of the β-adrenoceptors in homogenates and cryostat sections of the rat nasal mucosa. Competition experiments with the subtype selective antagonists $LK_{203-030}$ and $ICI_{118,551}$ demonstrated the presence of approximately 50% β_1- and β_2-adrenoceptors in homogenates as well as in cryostat sections. The agonist inhibition curves showed 30% high affinity sites. Autoradiograms demonstrated the presence of β_1- and β_2-adrenoceptors in the epithelium as well as in the glandular excretory ducts.

The pharmacological characteristics of α_1- and α_2-adrenoceptors in rat nasal mucosa have been described in **chapter 5 and 6.** Because of the small

number of α_1-adrenoceptors in the rat nasal mucosa, the characterization of these receptors was limited and the localization appeared to be impossible. The selective antagonist WB4101 demonstrated α_1-subtypes in rat nasal mucosa. The agonist inhibition curve had a monophasic character, indicating an homogeneous population of agonist binding sites. In first instance, the antagonist ^{3}H-Rauwolscine appeared to bind to α_2-adrenoceptor subpopulations. The appearance of two binding sites was probably due to an inappropriate definition of the specific binding, caused by a non-linear non-specific binding. Inhibition of the ^{3}H-Rauwolscine binding with the subtype selective antagonist prazosine suggested the presence of α_2-adrenoceptor subclasses. The agonist inhibition curve showed high and low affinity binding sites. In the presence of guanine nucleotides high affinity sites were converted into low affinity binding sites. Until now, localization of the α_2-adrenoceptors with in vitro autoradiography was not succesful.

Chapter 7 describes the characteristics of patients from whom we received a biopsy. The following parameters were involved: nasal symptoms, family history, X-rays of the sinuses, eosinophils in the blood and in the nose, serum IgE, skin- and RAST-tests. The patients were classified into a non-allergic and allergic group. Furthermore, the heterogeneous non-allergic group has been subdivided into controls, chronic sinusitis and vasomotor rhinitis patients. Only 30% of the biopsies could be used for biochemical experiments because no clear distinction between non-allergic and allergic patients could be made in every case and/or because of the limited amount of tissue.

A histological study of the nasal mucosa of non-allergic and allergic patients has been described in **chapter 8.** The in glycol methacrylate embedded specimens of the nasal mucosa of most patients showed various tissue components; epithelium and the lamina propria with glands, blood vessels and infiltrating cells. The AB/PAS staining indicated the presence of neutral or neutral and acidic glycoproteins in goblet cells as well as in the tubulo-alveolar glands. In specimens from allergic rhinitis patients basophilic cells were observed in the epithelium, whereas no basophilic cells could be observed in control individuals. An increased number of basophilic cells was observed in the lamina propria

of allergic rhinitis in comparison with non-allergic patients.
In **chapter 9** the hypothesis of changes in characteristics of muscarinic receptors in the nasal mucosa of allergic patients is addressed. The heterogeneous non-allergic group was subdivided into subgroups as described in chapter 7. In allergic patients, the equilibrium dissociation constant and density of muscarinic receptors were decreased in comparison with non-allergic or control individuals. No differences in agonist binding or coupling of this receptor to the G-protein could be observed in allergic patients. In vitro autoradiography demonstrated the presence of muscarinic receptors in the glandular acini of non-allergic and allergic patients. The increased sensitivity accompanied by a decreased receptor density may reflect the cholinergic nasal hyperreactivity in nasal allergy.
In **chapter 10**, the hypothesis of changes in characteristics of adrenergic, α_1-, α_2- and β-adrenergic, receptors in the nasal mucosa of allergic patients is addressed. The heterogeneous non-allergic group has been subdivided into the subgroups as described above. No significant differences in affinities or densities of α_1- as well as α_2-adrenoceptors could be demonstrated in allergic patients in comparison with non-allergic or control individuals. The β-adrenoceptor density, expressed per mg protein, was significantly reduced in allergic patients in comparison with controls. No changes in agonist binding or guanine nucleotide coupling could be observed in allergic patients. The subtype selective antagonist $IK_{203-030}$ demonstrated the presence of a homogeneous population of β_2-adrenoceptors in human nasal mucosa of both non-allergic and allergic patients. In vitro autoradiography showed the presence of β-adrenoceptors in the epithelium of both groups of patients. The decreased β-adrenoceptor density in nasal allergy may reflect a β-adrenergic abnormality in nasal allergy.

In conclusion, the rat can be used as an experimental model to develop and optimize radioligand receptor binding and in vitro autoradiographic assays for investigating the pharmacological characteristics and localization of various neuroreceptors in the nasal mucosa. Both techniques have been applied to human nasal mucosa in order to

investigate the supposed changes in the pharmacological characteristics of neuroreceptors in nasal allergy. The classification of patients into non-allergic and allergic patients as well as the subdivision of the non-allergic group into controls, vasomotor rhinitis and chronic sinusitis patients appeared to be of prime importance. An accompanying histological study helped to interpret the biochemical studies. The combination of radioligand binding studies and in vitro autoradiography indicated the presence of muscarinic acetylcholine receptors in the nasal glands and an homogeneous population of β_2-adrenoceptors in the epithelium of the nasal mucosa of non-allergic as well as allergic patients.

An increased sensitivity and a decreased number of muscarinic receptors have been demonstrated in the nasal mucosa of allergic patients in comparison with controls. These findings may reflect the cholinergic induced hypersecretion in nasal allergy. No differences in affinities or densities of α-adrenoceptors in the nasal mucosa of allergic patients in comparison with non-allergic individuals could be demonstrated. On the other hand, a decreased number of β-adrenoceptors have been demonstrated in allergic patients in comparison with controls, which may reflect a β-adrenergic abnormality. The small shifts in affinity and receptor density may reflect the cholinergic hyperreactivity and a β-adrenergic abnormality in nasal allergy, but are probably too small to explain the complex allergic reaction.

Nasale hyperreactiviteit bij allergische rhinitis wordt mogelijk veroorzaakt door een disbalans in de autonome regulatie van het neusslijmvlies door bijv. veranderingen in eigenschappen van neuroreceptoren. In deze studie werden radioligand receptorbinding en in vitro autoradiografische technieken ontwikkeld om de receptordichtheden, gevoeligheden, subtypen, receptor-effector koppelingen en lokalisatie van neuroreceptoren in het neusslijmvlies te bepalen. In eerste instantie werd de rat als proefdiermodel gebruikt, zodat de geringe hoeveelheid humaan weefsel geen beperkende factor zou zijn. Vervolgens werden beide technieken toegepast op humaan neusslijmvlies van niet-allergische en allergische patienten.

Hoofdstuk 1 bevat een overzicht van de anatomie, fysiologie en autonome regulatie van het neusslijmvlies en bespreekt een aantal farmacologische en biochemische eigenschappen van verschillende neuroreceptoren. Tevens wordt aandacht gegeven aan nasale hyperreactiviteit.

In **hoofdstuk 2 en 3** worden de farmacologische karakterisatie en lokalisatie van muscarine cholinerge receptoren in het neusslijmvlies van de rat beschreven. Op basis van de selectieve antagonisten AF-DX-116 en HHSiD werden de muscarine receptoren in het neusslijmvlies gekarakteriseerd als M_3 receptoren. Na toevoeging van diverse protease-remmers, bleek het mogelijk hoge en lage affiniteitsbindingsplaatsen aan te tonen bij de verdringing van de antagonist binding met agonisten. In vitro autoradiografie toonde de aanwezigheid van muscarine receptoren in de acini en in geringe mate in de afvoergangen van de klieren.

Hoofdstuk 4 beschrijft de farmacologische karakterisatie en lokalisatie van de β-adrenoceptoren in homogenaten en vriescoupes van het neusslijmvlies van de rat. Uit verdringingsexperimenten met de selectieve antagonisten $LK_{203-030}$ en $ICI_{118,551}$ bleken ongeveer 50% β_1- en β_2-adrenoceptoren aanwezig in homogenaten maar ook in vriescoupes van het neusslijmvlies. De agonist verdringingscurve toonde 30% hoge affiniteitsbindingsplaatsen. Autoradiogrammen toonden de aanwezigheid van β_1- en β_2-adrenoceptoren in het epitheel en in de afvoergangen van de

klieren.

In **hoofdstuk 5 en 6** worden de farmacologische kenmerken van α_1- en α_2-adrenoceptoren in het neusslijmvlies van de rat beschreven. De geringe hoeveelheid α_1-adrenoceptoren in het neusslijmvlies beperkte de karakterisatie en maakte de lokalisatie onmogelijk. De selectieve antagonist WB4101 toonde α_1-subtypen in het neusslijmvlies van de rat. De verdringingscurve van de antagonist binding met agonist verliep monofasisch, wijzend op een homogene populatie agonistbindingsplaatsen. De antagonist ^{3}H-Rauwolscine bleek in eerste instantie te binden aan 2 subpopulaties van α_2-adrenoceptoren. Het optreden van twee bindingsplaatsen werd mogelijk veroorzaakt door een onjuiste definitie van de specifieke binding wegens een niet-lineaire aspecifieke binding. Inhibitie met de selectieve antagonist prazosine suggereerde de aanwezigheid van α_2-subtypen. De agonist inhibitie curve toonde zowel hoge als lage affiniteitsbindingsplaatsen. Na toevoeging van guanine nucleotiden werden de hoge affiniteitsbindingsplaatsen omgezet in lage affiniteitsbindingsplaatsen. Tot op heden bleek het niet mogelijk α_2-adrenoceptoren te lokaliseren m.b.v. de in vitro autoradiografie.

Hoofdstuk 7 beschrijft het klinische beeld van de patienten, van wie een neusbiopt verkregen werd voor het humane deel van het onderzoek. Hierbij werden de volgende parameters gehanteerd; symptomen, familie achergronden, foto's van de sinus holten, eosinofiele cellen in het bloed en in de neus, IgE gehalte in het serum, RAST en huid-testen. De patienten werden geklassificeerd in een niet-allergische en allergische groep. Vervolgens werd de heterogene niet-allergische groep verdeeld in controle individuen, chronische sinusitis en rhinitis vasomotoria patienten. Slechts 30% van de verkregen biopten bleek bruikbaar voor biochemische studies wegens de geringe hoeveelheid verkregen weefsel of omdat niet altijd een duidelijk onderscheid gemaakt kon worden tussen niet-allergische en allergische patienten.

Een histologische studie van de neusbiopten van niet-allergische en allergische patienten is beschreven in **hoofdstuk 8.** De plastic coupes van het neusslijmvlies van de meeste patienten toonde diverse weefselcomponenten: epitheel en de lamina propria met klieren, bloedvaten en bindweefsel. Neutrale en zure glycoproteinen bleken aanwezig in de

slijmbekercellen van het epitheel en in de tubulo-alveolaire klieren. In coupes van allergische patienten werden basofiele cellen (mest cellen), waargenomen in het epitheel, terwijl deze cellen niet werden waargenomen in controle individuen. Tevens werd een verhoogd aantal basofiele cellen waargenomen in de lamina propria bij allergische patienten t.o.v. controle individuen.

In **hoofdstuk 9** wordt de hypothese van veranderingen in de farmacologische kenmerken van muscarine receptoren in het neusslijmvlies van allergische patienten getoetst. De heterogene niet-allergische groep werd verdeeld in subgroepen zoals hierboven beschreven. In allergische patienten was het aantal muscarine receptoren verlaagd en de gevoeligheid verhoogd t.o.v. niet-allergische patienten en controle individuen. Geen veranderingen in agonist binding en in de koppeling van de receptor aan het G-eiwit konden worden waargenomen in allergische patienten t.o.v. niet-allergische individuen. In vitro autoradiografie toonde de aanwezigheid van muscarine receptoren in de acini van de klieren in niet-allergische en allergische patienten. De verhoogde gevoeligheid en een verlaagd aantal muscarine receptoren reflecteert mogelijk de cholinerge hyperreactiviteit bij allergische patienten.

In **hoofdstuk 10** werd de hypothese van veranderingen in kenmerken van adrenerge receptoren, α_1-, α_2- en β-adrenoceptoren, in het neusslijmvlies van allergische patienten getoetst. De heterogene niet-allergische groep werd gesplitst zoals hierboven beschreven. Geen veranderingen in gevoeligheden en/of receptordichtheden van α-adrenoceptoren konden worden waargenomen in allergische patienten t.o.v. niet allergische en controle individuen. De β-adrenoceptor dichtheid, uitgedrukt per mg eiwit, was significant verlaagd in allergische patienten t.o.v. controle individuen. Geen veranderingen in agonist binding of koppeling van de β-adrenoceptor aan het G-eiwit werden waargenomen in allergische patienten t.o.v. niet-allergische patienten.

De combinatie van radioligand binding met de selectieve antagonist $LK_{203-030}$ en de in vitro autoradiografie toonde een homogene populatie β_2-adrenoceptoren in het epitheel van het neusslijmvlies van zowel allergische als niet-allergische patienten. Een verlaagd aantal β-adrenoceptoren in het neusslijmvlies van allergische patienten

reflecteert mogelijk een β-adrenerge abnormaliteit bij allergische patienten.

Samengevat kan gesteld worden, dat de rat een goed proefdiermodel is om radioligand receptor binding en in vitro autoradiografische technieken te ontwikkelen en te optimaliseren. Beide technieken konden worden toegepast op humaan neusslijmvlies om mogelijke veranderingen in neuroreceptoren bij nasale allergie te onderzoeken. De klassificatie van patienten in niet-allergische en allergische individuen, maar ook de onderverdeling van de heterogene niet-allergische groep in controle, chronische sinusitis en rhinitis vasomotoria patienten bleken van primair belang. De data van de biochemische studies konden beter geïnterpreteerd worden door hiernaast een histologische studie uit te voeren. De combinatie van radioligand receptorbinding studies en in vitro autoradiografie toonden de aanwezigheid van muscarine receptoren in de klieren en een homogene populatie β_2-adrenoceptoren in het epitheel van het neusslijmvlies van zowel niet-allergische als allergische patienten. De verhoogde gevoeligheid en de verlaagde dichtheid van de muscarine acetylcholine receptoren in het neusslijmvlies van allergische patienten in vergelijking met controle individuen reflecteert mogelijk de cholinerge hyperreactiviteit van allergische patienten. Geen verschillen in affiniteit en/of dichtheden van α-adrenoceptoren konden worden aangetoond in het neusslijmvlies van allergische patienten t.o.v. niet-allergische individuen. Het aangetoonde verlaagde aantal β-adrenoceptoren in het neusslijmvlies van allergische patienten t.o.v. controle individuen daarentegen, reflecteert mogelijk een β-adrenerge abnormaliteit in allergische patienten. Deze veranderingen in affiniteit en receptordichtheid verklaren mogelijk de cholinerge hyperreactiviteit en een β-adrenerge abnormaliteit in allergische rhinitis patienten, maar zijn waarschijnlijk te gering om de complexe allergische reactie te verklaren.

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De schrijfster van dit proefschrift werd geboren op 15 december 1960 te Nijmegen. In 1979 werd het diploma Gymnasium-B behaald aan de Nijmeegse Scholengemeenschap.

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